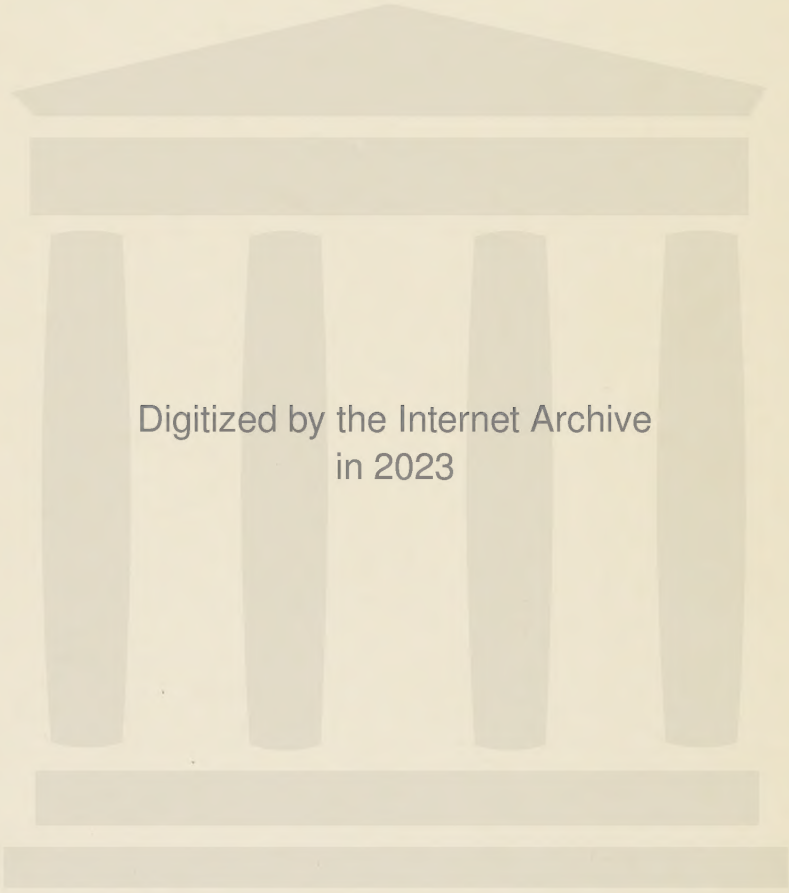
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RHIZOCTONIA CROCORUM (PERS.) DC. AND R. SOLANI KÜHN (CORTICIUM VAGUM B. & C.), WITH NOTES ON OTHER SPECIES

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The form genus *Rhizoctonia* was established in 1815 to include two parasitic species, both characterized in part by the production of a mat of violet mycelium investing the affected roots or other submerged members. The serious root diseases due to these organisms (later included in one species) have received consideration by many mycologists since that time. The demonstration is comparatively recent, however, that several important types of root and certain stem and other diseases of a variety of hosts are induced by two or more related species of this genus.

The literature of Rhizoctonia diseases has grown enormously in the past fifteen years, yet some unnecessary confusion and difference of opinion exist regarding the two main species or groups of species and their distribution and relation to disease in plants. This is in part due to the lack of comparative study and to the neglect or inadequacy of herbarium material. It seems well, therefore, to present a conspectus of the investigations relating to this subject, and to include such comparative data as are available.

In coöperation with Mr. F. C. Stewart of the New York (Geneva) Agricultural Experiment Station, I undertook, in 1898, a general study of the relation of *Rhizoctonia* to plant diseases in America. This joint investigation followed two

independent studies, one of a serious root disease of the sugar beet, the other of a destructive stem rot of the carnation. A preliminary report upon the investigations relating to *R. Solani* Kühn was published ('01), and it was arranged that in the further work one of us would undertake the morphological, cultural, and taxonomic aspects of the study, and that the other would assume responsibility for all cross inoculation and field work. Unfortunately for this purpose a change of position on the part of one of us and the demands of other work necessitated the abandonment of the plan as proposed. It is to be regretted particularly that the systematic inoculation experiments which had been carried forward for two seasons could not be continued and published. It is understood, however, that an extensive study in the relations of the culturable forms on different hosts has been carried forward both by cultural and inoculation experiments at the University of Illinois by Dr. George L. Peltier, who has already presented a preliminary report ('15), on the subject. It is mainly a general account of the diseases with notes on comparative morphology that I am able to include, but it is hoped that this may serve to clear up the more obvious difficulties and to suggest some problems requiring special investigation.

The writer wishes to acknowledge the assistance, mentioned in the text, of many mycologists who have furnished material during the progress of these studies, and especially the coöperation of Mr. F. C. Stewart, who contributed many of the American hosts during the earlier studies. To Prof. E. A. Burt I am also indebted for suggestions.

The Violet Root Felt Fungus, Rhizoctonia Crocorum (Pers.) DC.

Early pathological studies

The first mention of a plant disease which may be referred with certainty to *Rhizoctonia* as the causal agent is an important paper by Du Hamel (1728) read before the Paris Academy. In this paper he gives a careful description of a fungous disease of *Crocus sativus* (saffron) occurring in France. His description of general pathological features

leaves little to be desired, and one cannot mistake the fact that he was discussing the disease, later known to be due to *Rhizoctonia Crocorum*. He does not describe the more minute morphological features, but discusses the macroscopic appearance of the mycelium and sclerotial stages with such completeness that no doubt remains concerning the identity of the fungus. The illustration included would likewise confirm the description. He regarded the sclerotium, "tubercule," as the fruit body of a fungus allied to the truffles, and to this special form of body, assumed to bear the organs of reproduction, he gave the name "tuberoides." He likewise determined that a similar fungus is the cause of a disease found upon the roots of *Sambucus Ebulus, Coronilla varia, Ononis spinosa, Muscari* sp., and perhaps other plants.

It was more than fifty years later that Fougeroux de Bondaroy (1785), discussing primarily a disease of the saffron known as "tacon" gives further notes on the "mort du safran," recording the occurrence of this disease on asparagus when following (in the same soil) diseased crocus.

After a further considerable lapse of time De Candolle (1815) made a careful study of the pathology of a similar alfalfa (*Medicago sativa*) disease in the vicinity of Montpellier, but known throughout France. This led to the establishment of the genus *Rhizoctonia* as noted later. It is necessary to the pathological account to note here, however, that he recognized two species, *R. Crocorum* DC., primarily inhabiting the crocus, and *R. Medicaginis* DC., on the alfalfa and other hosts. He did not follow the development of the fungus on the saffron, where host characteristics render somewhat obscure the appearance of the fungus; and so for a long time the continuous violet felt of mycelium was associated primarily with *R. Medicaginis*.

Among other diseases of the carrot and beets in Germany, Kühn ('58) found typical rots of these root crops, accompanied in both cases by a red-violet mycelium with other characteristics indicating the alfalfa organism. He identified the fungus as *R. Medicaginis* and thus established the greater importance of Rhizoctonia diseases, and greatly extended the

range of the fungus. He found a somewhat similar disease of the potato, but clearly distinguished the fungus as another species, as further indicated in another part of this paper.

Chief among those who extended our knowledge of the pathology and distribution of the violet root felt fungus was Rostrup ('86), who observed the fungus in Denmark and described its effects on various hosts.

EARLY TAXONOMIC AND MORPHOLOGICAL ACCOUNTS

The fungi belonging to the genus *Rhizoctonia* received attention taxonomically from the earliest mycologists. Brief references should be made to the works of some of those who have presented synopses of the genus or who have contributed to the solution of the problem regarding the taxonomic position of these fungi. Bulliard (1791) evidently based his description of species upon the observations and data of Du Hamel and de Bondaroy; emphasizing therefore the sclerotium as the fruit body, and believing it homologous with the truffle he gave to this fungus on *Crocus sativus* the name *Tuber parasiticum.* He contributed nothing further to the morphology of the species. Persoon (1801) did not accept Bulliard's disposition of the fungus, but named it *Sclerotium Crocorum,* and gave a diagnosis which, while based on the observations of the earlier writers, did not confuse the sclerotium with a true fruit body.

De Candolle (1815[a]), in his first taxonomic discussion employed Persoon's name for the fungus, and then, after giving the characteristics and parasitism of the species on alfalfa more careful attention, he established (1815, 1815[b]) the genus *Rhizoctonia* to include two species, *R. Crocorum* DC. on crocus and other hosts and *R. Medicaginis* DC. on alfalfa. It will be noted that he adopts Persoon's specific name for the crocus fungus. De Candolle also considers a doubtful species, *R. Mali,* reported on apple.

Nees (1816) placed the crocus fungus in *Thanatophytum* under the name *T. Crocorum.* Fries (1823) assigns *Rhizoctonia* to the *Sclerotiaceae* just following his extensive genus *Sclerotium.* It is important to note, since Fries' work has been

made the starting point for mycological nomenclature, that he designates three species in the following order, (1) *R. Crocorum* DC., (2) *R. Medicaginis* DC., and (3) *R. muscorum* Fr., also giving *R. Mali* DC. among *species ignota.* The descriptions of the two species first mentioned leave no doubt that he is here defining the violet root felt fungus of crocus and of alfalfa. Moreover, Fries recognized *Sclerotium Crocorum* Pers. as a synonym of *R. Crocorum* DC. So far as has been ascertained no specimens of these species which he examined are still in existence. Link (1824) excluded the doubtful forms, added a species *R. strobilina,* and otherwise left the genus as constituted by De Candolle. Duby (1830) included among the species *Rhizoctonia Allii* Graves, arranging the genus close to *Sclerotium* in the *Scleroteae* of *Lycoperdaceae.* Fries later included in this genus *R. Batatas* Fr. on *Ipomoea Batatas* from America.

The most complete mycological account of the genus *Rhizoctonia* is that given by L. and C. Tulasne ('62). They reduce *R. Crocorum* DC. and *R. Medicaginis* DC. to a single species to which they apply a new name, significant of the appearance of the fungus, *R. violacea* Tul. This reduction to a single form was made after a most careful morphological study of the fungus in all stages. From the accurate descriptions and the excellent illustrations it is clear that they had under consideration material referable to the names above. The Tulasne brothers also refer to other species insufficiently known, as follows: *R. Allii* Graves, *R. Batatas* Fr., and *R.* (?) *Mali* DC. They were inclined to the view that the affinities of the genus would be found to be with the *Ascomycetes,* and, in fact, they considered certain minute cushions of hyphae, referred to in detail later, as immature perithecia. Successively, therefore, attention was drawn by mycologists (1) to the sclerotium as a fruit body (Du Hamel and Bulliard), (2) to the sclerotium as a sterile structure (Persoon), (3) to the strand-like habit of the mycelium (De Candolle), and (4) to the minute cushion-like sclerotia as suggesting perithecia (Tulasne, L. and C.).

NAME, SYNONYMY, AND MATERIAL EXAMINED

Since the investigations of the brothers Tulasne many mycologists have studied the violet root felt fungus on its various hosts, especially on crocus, alfalfa, and certain root crops. There is general, though not complete, agreement in confirmation of the view that the crocus and the alfalfa forms are identical, and that this species, *R. Crocorum*, occurs on numerous hosts. I shall indicate later some of the morphological details in which the two forms agree and give other evidence supporting the view of a single species. For the present it is necessary to anticipate this evidence in order to state that until a perfect stage is definitely established, it would appear that the correct designation of the violet fungus is *Rhizoctonia Crocorum* (Pers.) DC. As noted above, the specific name applied by Persoon was adopted by De Candolle when he established the genus. This name, perhaps unfortunately, has priority over *R. Medicaginis* DC. in that it is mentioned first by Fries (1823). Though necessary, it may seem unwise to call the fungus *R. Crocorum*, inasmuch as it is far more widely distributed on alfalfa; and, furthermore, because its dicotyledonous hosts are more numerous. *R. violacea* would be a most appropriate descriptive name, but it is obvious that this also would not conform to the rules. The following provisional synonymy has been collated:

Tuber parasiticum Bull. (1791),
Sclerotium Crocorum Pers. (1801),
Rhizoctonia Crocorum DC. (1815),
Rhizoctonia Medicaginis DC. (1815),
Thanatophytum Crocorum Nees. (1816),
Tuber Croci Duby (1830),
Rhizoctonia Rubiae Dcne. (1837),
Rhizoctonia Dauci Rabenh. (1859),
Rhizoctonia violacea Tul. (1862),
Rhizoctonia Asparagi Fckl. [non Fr.] (1869),
Hypochnus violaceus Eriks. (1913).

The identity of *Rhizoctonia Crocorum* DC. and *R. Medicaginis* DC. suggested by the brothers Tulasne ('62) and accepted by most taxonomists, has been confirmed by a study

of all the material I have been able to examine, and there is included below a list of the material identified as *Rhizoctonia Crocorum* (Pers.) DC.

Exsiccati: *Rhizoctonia Medicaginis* DC., Linhart, Fung. Hung. Fasc. 4: *400; Rhizoctonia Dauci* Rabenh., Rabenhorst, Herb. Mycolog. Fasc. 1: *74.* (*Helminthosporium rhizoctonum* Rabenh.); *Rhizoctonia Solani* Kühn, De Thuemen, Myc. Univ. Cent. 18 : *1797.*

European collections: (1) Material from Prof. Delacroix, Paris, 1901, as follows: on sugar beet; on sugar beet, obtained by inoculation from diseased beet; on potato; on potato, by inoculation from affected beet; on crocus; on crocus, by inoculation from affected beet; on alfalfa; on *Onobrychis sativus;* on asparagus; and on asparagus, by inoculation from diseased beet. (2) On crocus from bulb gardens, Pithiviers, France, 1901. (3) From Prof. Aderhold, Proskau, Germany, 1899, on carrot and on root of young apple tree. (4) From Prof. Sorauer, Berlin, 1900, on potato and on asparagus. (5) From Herr Weigand, Helmitzheim, Bavaria, 1899, on alfalfa. (6) From Prof. v. Tubeuf, Munich, 1899, on sugar beet. (7) From Prof. Hartig, Munich, on roots of young conifer. (8) From Prof. Cugini, Modena, Italy, 1899, on alfalfa. (9) Material which the writer was able to obtain fresh near Munich, 1905, on sugar beet and alfalfa.

In 1901 the writer was unable to find in the Kew Herbarium or in Paris any type material, and none was found in Montpellier in 1905.

American collections: (1) From Mr. P. W. Graff, Manhattan, Kansas, 1911, on alfalfa. (2) From Mr. F. D. Bailey, Laurel, Oregon, (sent by Dr. G. L. Peltier, Univ. of Ill.) 1915, on potato.

DISTRIBUTION

In Europe the violet root felt fungus is in general widely distributed, but its occurrence now and then in epidemic form on some one host would appear to indicate some locality or race influence. On *Crocus sativus* the fungus has been reported from France chiefly; on asparagus, more frequently from France, Belgium, and Italy; on *Medicago sativa* it would

seem to occur more commonly from southern France eastward to Bavaria and Hungary and southward to the Mediterranean. No information is available with respect to its occurrence in Russia. On the fleshy root crops and on the potato the fungus has often been reported from central France and Germany northward through Denmark, Norway and Sweden, and also on the sugar beet in Italy. In Denmark it appears to be found oftener on species of *Trifolium* than on alfalfa.

The root felt disease is certainly not unknown to market gardeners and others throughout England, yet there are relatively few references to it in pathological literature. It would appear that Güssow has observed the fungus in England, for in speaking of diseased tubers from a farm in Essex he says, "They were covered with a dull reddish-brown webbing, which was raised into numerous points, as if grains of sand were below it," but in view of his reference in the same article to the commoner potato fungus no definite statement should be made. Salmon's account ('08) of the disease of seakale, described as "a felted mass of violet spawn or mycelium," evidently refers to this species.

In the United States *R. Crocorum* was first reported from Nebraska by Webber ('90) on lucerne. He states that it was rare in the Nebraska flora at that time. Heald ('06) lists the fungus as among disease-producing organisms prevalent in Nebraska during 1905. The record is as follows: "Root rot. *Rhizoctonia violacea* Tul. reported from a single locality: Platte County. Not common in that region." The complete observations made in 1906 were not reported until later, in which account, however, Heald ('11) fails to make note of Webber's earlier report of its occurrence. Freeman ('08) refers to the fungus as the cause of a well established disease of alfalfa in Kansas, and a specimen received by the writer in 1911 from that state indicates that it is identical with the European fungus. More recently it has been mentioned by Gandara ('10), and the inference is that it is found on alfalfa in Mexico. The first occurrence on potato in America is from a locality in Oregon (Bailey, '15). No well authenticated instance of the occurrence of this fungus in South America,

Australia, Asia, or Africa has come to my attention, yet the distribution of alfalfa growing throughout the world and the frequent interchange of seed might suggest that the distribution of the organism may be found to be much more general than is reported. It should be mentioned that Shaw ('13) reports the fungus from India, but he has obviously been misled regarding the fungus concerned, as will be shown later.

Du Hamel represented the violet root fungus as prevailing under a variety of soil conditions, but electing dry, gravelly, and acid localities. It is reported by the brothers Tulasne that while wet weather may give the fungus an advantage, still it is found in the driest situations permitting crop growth. In central Germany Kühn's studies led to the suggestion that on root crops and potatoes it is found more frequently in low and stagnant places. Frank and Comes concur in this view. The writer was able to observe the fungus in the vicinity of Munich in 1905 and in the fields examined, it was found under conditions which appeared to be favorable for the growth of the host. The very general occurrence of the fungus in southern Europe, especially in southern France and Italy, would seem to indicate that excessive moisture is not always an important factor. At the same time the fungus is of frequent occurrence in Scandinavia. It is not reported as one of the more serious diseases of any host in England. In the more humid regions of the eastern United States it is unknown, while two of the localities from which it has been reported are regions of lower humidity and lesser rainfall.

HOST PLANTS AND GENERAL SYMPTOMS

There is every reason to believe that the number of host plants for *Rhizoctonia Crocorum* is much greater than has been reported. The fungus has been observed upon many economic plants; and it has been reported in the agricultural press of Europe as occurring upon a variety of weeds, but these references are not always definite. Eriksson has made some observations regarding the plants attacked when culti-

vated in soil from a carrot field known to be infected, and the following weed hosts are noted: *Stellaria media, Myosotis arvensis, Galeopsis Tetrahit, Erysimum cheiranthoides, Urtica dioica.*

This would indicate that a careful study of any epidemic would confirm the view that the number of hosts is considerable. The following is a list by families of the host plants which have been reported in the more accessible literature:

Pinaceae
- Abies pectinata
- Picea alba
- Picea excelsor
- Pinus Laricio
- Pinus montana

Liliaceae
- Asparagus officinalis
- Crocus sativus
- Lilium sp.
- Muscari sp.
- Narcissus sp.
- Tulipa sp.

Urticaceae
- Ficus silvatica
- Humulus Lupulus
- Urtica dioica

Polygonaceae
- Rumex crispus

Chenopodiaceae
- Beta vulgaris
- Chenopodium album

Caryophyllaceae
- Spergula arvensis
- Stellaria media

Cruciferae
- Brassica campestris
- Brassica Rapa
- Crambe maritima

Rosaceae
- Crataegus oxyacantha
- Pyrus Malus

Leguminosae
- Anthyllis vulneraria
- Coronilla varia
- Medicago lupulina
- Medicago sativa
- Melilotus alba

Leguminosae
- Onobrychis sativa
- Ononis spinosa
- Ornithopus sativus
- Phaseolus sp.
- Trifolium hybridum
- Trifolium pratense
- Trifolium repens
- Vicia Faba

Geraniaceae
- Geranium pusillum

Rutaceae
- Citrus Aurantium

Vitaceae
- Vitis sp.

Umbelliferae
- Daucus Carota
- Erysimum cheiranthoides
- Foeniculum vulgare
- Pastinaca sativa

Oleaceae
- Ligustrum vulgare

Convolvulaceae
- Convolvulus arvensis

Boraginaceae
- Myosotis arvensis

Labiatae
- Galeopsis Tetrahit

Solanaceae
- Solanum tuberosum

Rubiaceae
- Rubia tinctoria

Caprifoliaceae
- Sambucus Ebulus

Compositae
- Taraxacum officinale
- Sonchus arvensis
- Sonchus oleraceus

The difficulty in giving an accurate list of hosts compiled from the literature is, however, a serious one, since one cannot be certain that all the observations are carefully made. Again, some mycologists do not distinguish the two species of *Rhizoctonia* here discussed; thus Salmon ('08), after describing an interesting disease of seakale with all the characteristics of *R. Crocorum,* goes on to refer to carnation stem rot, damping off, and other diseases as if they were induced by the same fungus, doubtless, however, intended to have reference to another related fungus.

Regarding the above-ground symptoms of affected plants, it may be said that they are not striking, and were it not for the characteristic dead area in the field it would not be an easy matter to designate slightly affected plants. Generally, there is in alfalfa evidence of yellowing, sometimes marked chlorosis, while in beets and carrots there is merely a paler appearance of the foliage, followed by wilting. The critical period for affected alfalfa is usually about the time of the second cutting, and at this time considerable wilting may occur without preliminary indications of lack of health. In these main effects the disease is remarkably similar to the Texas root rot of cotton, alfalfa, and other plants. The unmistakable symptom is the relatively sudden dying of the plant affected.

The disease is generally though not necessarily fatal. Even a plant so susceptible as the alfalfa may recover from early injuries, usually with the loss of the tap root. Under certain conditions the disease incites the development of adventitious roots,—which may be a factor in recovery. The progress of the disease in the field is radial, and during the first year especially, circular dead areas mark its presence. The spread of the fungus during the season may be from a few feet to several rods. After the first year or two, considerable areas irregular in outline may be involved.

MYCELIUM AND SCLEROTIA

It would be difficult to confuse the mycelium of the violet root felt fungus with any other species, for when one is

familiar with it in the different stages of development it is at all times an organism with striking characteristics. Such differences in appearance as may be found in comparable stages on the various hosts may be regarded as causally related to the host substratum, or, at least they may be so regarded until adequate morphological differences or contrasting physiological relations are established. The general appearance of affected roots of asparagus, carrot, beet, or alfalfa are well expressed in some of the common names applied, such as red root, root felt disease, violet fungus, etc.

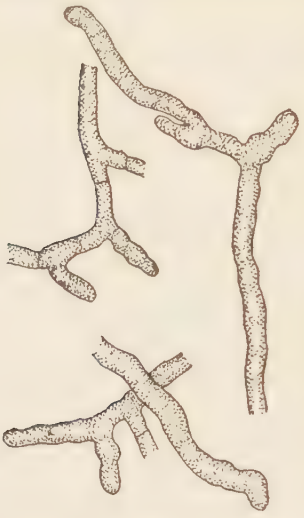

Fig. 1. *Rhizoctonia Crocorum:* Young hyphae.

With sufficient time for abundant growth the fungus completely invests the root or root system with a mantle, weft, or mat of hyphae of characteristic color. In the early stages of growth on the root the mycelium is pale buff to violaceous, but when the root is completely invested, the mycelium is red-violet to violet-brown, and always violet-brown with age or when densely matted. The numerous small darker papillae or "minute sclerotia" in the mantle of mycelium are in reality cushion-like mycelial bodies described later.

In the following description the writer will not attempt to follow all changes in the development of the various mycelial conditions, but will endeavor to give briefly those developmental features of greatest interest and those diagnostic characteristics which may be applied to most herbarium material. For further morphological details the accounts of L. and C. Tulasne ('62) and Prillieux ('91) should be consulted.

The external, general hyphae are more or less different in form and appearance with age. The younger hyphae are usually dilutely violaceous with a pigment which may be decolorized by the application of acidulated water. The pro-

toplasm is dense towards the tips of branches and vacuolated farther away. The hyphae are somewhat flexuous, branched (sometimes closely), with the branches arising at right angles to the main hypha, and with a partition wall laid down at not over 10 μ distant (fig. 1). With age the hyphae become rigid, somewhat less in diameter, 4–8 μ, the branching is distant, and these branches readily break off at the first partition wall (fig. 2). At the point of union the diameter is uniform with the main hypha. The partition walls are distant,

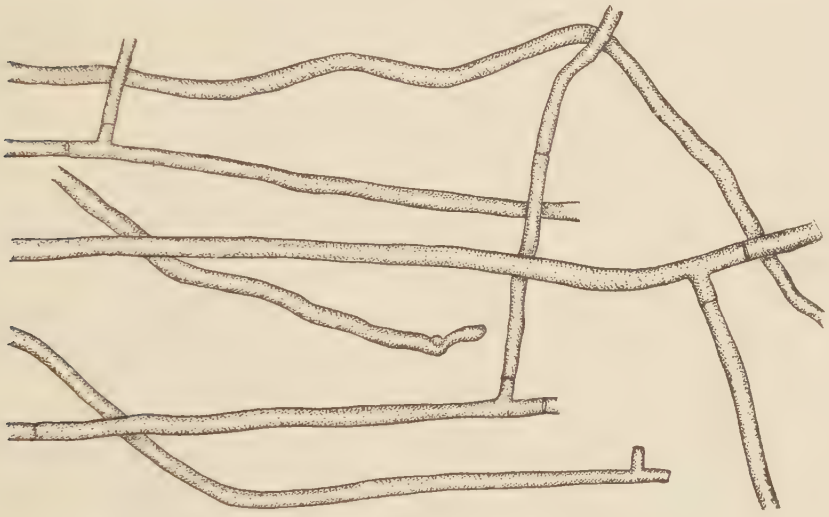

Fig. 2. *Rhizoctonia Crocorum:* Mature root-investing hyphae.

often 120–200 μ apart. The walls now possess the violet-brown pigment and in the lumen little or no protoplasm is observable.

The internal mycelium is likewise branched, septate, often associated into loose strands, passing between the cells or traversing them. In the early stages of the disease, so far as reported, these internal hyphae are nearly colorless; Prunet reports that there are sometimes areas of brown mycelium in the attacked tissues, and this I find particularly true of asparagus. The internal hyphae are generally of less diameter than those constituting the external mat.

Disregarding for the time the small cushions already men-

tioned, the hyphae constituting the external mantle may be uniformly distributed, as is the case usually when the fungus attacks fleshy roots or tubers, or they may also form a number of aggregates having the appearance of loose or root-like strands. The strands are developed later rather than early in the progress of the disease. They are conspicuous on such hosts as alfalfa and sainfoin. These strands course along the whole root system; they also pass out into the soil, apparently beyond the minutest rootlets, and doubtless attack plants in the vicinity. Upon the larger strands sclerotia may be formed, and thus the sclerotia are connected with the mantle of hyphae.

Infection Cushions.—Small stromatic bodies distributed amongst the hyphae were noted by several of the early observers. Kühn ('58) calls special attention to them on the carrot and the potato. The brothers Tulasne ('62) studied and described them in some detail and came to the conclusion that these were the early stages in the development of the perithecial form. Search for the reproductive phase was in this way transferred from the sclerotium to the bodies in question. Sorauer ('86) among others accepted the view of the perithecial nature of this structure. Prillieux ('91) seems to have been the first to point out that the "corps miliaires," as he termed them, are in reality special mycelial cushions having the important function of effecting the penetration of the host. He regarded them as the main, if not the sole, seats of tissue invasion, and his studies included a comparison of these bodies and of the penetrating strands in alfalfa, sugar beet, and crocus. After mentioning these cushions as one type of sclerotia, Prunet designates them more specifically as minute "corps noirâtres," .2 to 1.2 mm. in diameter with a brown hyphal cortex and a colorless medullar. He indicates that these as well as the larger sclerotia send out filaments which enter the soil and extend the fungus. These bodies have also been figured by Bailey ('15) in the case of the occurrence of the fungus on the potato in Oregon and particularly well by Salmon and Crompton ('08, pl. 25). The writer is of the opinion that Prillieux's notion is in general correct,

and while they are not the only means of penetration they are most important in this connection.

The hosts upon which the writer has had the opportunity to examine the infection cushions in best condition are alfalfa, carrot, and asparagus. The cushions are distributed over infected roots, often 1 mm. apart in alfalfa, .5 mm. in carrot, and 3 mm. in asparagus. The external hyphae are for the most part similar to those of the general mycelium, but there occur also branches in which the cells are short and swollen, sometimes resembling a short chain of spores. This form of hypha may have given the suggestion of a conidial stage (see Kühn ('58), Sorauer ('86), and others. The medullary portion of younger cushions is made up of finer, almost colorless hyphae, and it is this type which enters—strand-like—the cortical tissues of the root, destroying particularly the cambium and younger phloem regions. In the later stages of development it will be found that the cushions seem to extend considerably into the cortex, and more of the hyphae are colored.

In this connection it is well to call attention briefly to some gross changes in the affected roots. By the time the host (alfalfa) reaches the critical stage, the bark slips readily from the root. The disintegration may continue further, however, through the spread of the fungus to the medullary rays and all other parenchyma, so that the root shreds or crumbles when lifted. The late stages of destruction may be assisted by saprophytic organisms. It is difficult to determine if the fungus continues its growth for a short time after the death of the root. At any rate, the fungus rapidly disappears with the further decay of the roots.

In the case of asparagus the cushions are largely superficial and the main affected tissues are beneath the shell of thick-walled cells constituting the periphery of the host. In the carrot the invading strands are large, and the host cells in the vicinity rapidly collapse and darken. I have been fortunate in obtaining affected asparagus roots at intervals after the disease had run its course. In no case could any evidences of spore forms be found which gave promise of genetic connection. On the contrary, the fungus gradually disappears,

first the mantle of mycelium, and then the cushions, so that when the root is reduced to a mere shell there are only vestiges of the cushions remaining.

Sclerotia.—The true sclerotia are flattened or rounded bodies varying in diameter from a few millimeters to several centimeters. When mature they are of a deep violet-brown

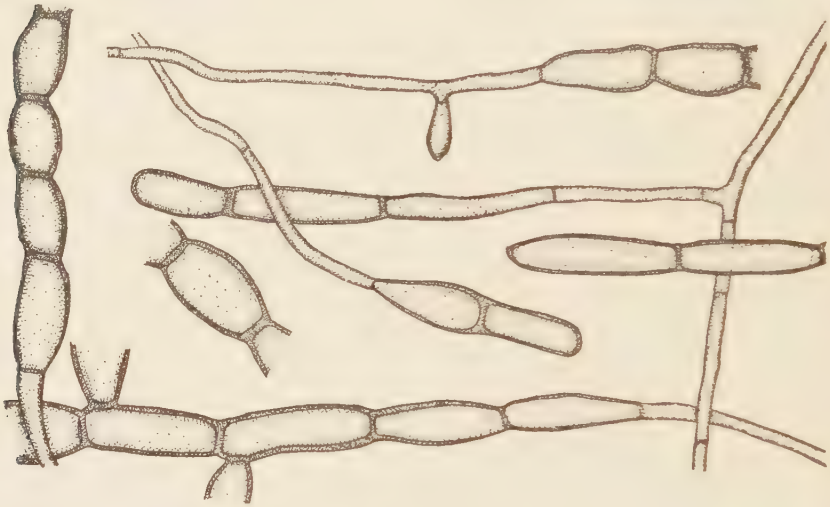

Fig. 3. *Rhizoctonia Crocorum:* Cells characteristic of the tufted growth covering the surfaces of the large sclerotia and to a certain extent of the "infection cushions."

and are thickly clothed with a persistent velvety felt, externally of the same color as the root-investing hyphae, but darkening further in. Among the surface hyphae of the sclerotia as well as of the "infection cushions" are found chains of enlarged cells (fig. 3) quite distinct from the enlarged cells of *R. Solani.* The sclerotia, as noted previously, are always connected with the root felt by large hyphal strands. In the saffron disease the sclerotia are formed both in contact with the shriveling bulb and also in the adjacent soil. On affected alfalfa roots they often occur below, and in the angles of, the larger branches, but often one finds no sclerotia in immediate contact with the host. In connection with diseased carrots, beets, and potatoes, they are not so frequent, unless perhaps they are then formed at greater

distances from the plant. Most herbarium material, unfortunately, with the exception of crocus specimens, does not include sclerotia.

In section a sclerotium consists of fairly compact tissue made up of cells often considerably branched and sometimes curiously lobed (fig. 4).

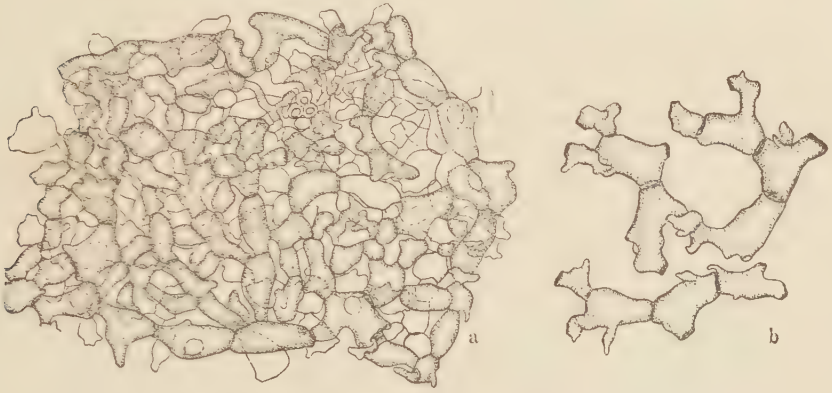

Fig. 4. *Rhizoctonia Crocorum:* a, from a section of a large sclerotium; b, extreme forms of cells isolated from a macerated sclerotium.

SUGGESTIONS REGARDING THE PERFECT STAGE

It has been noted that Du Hamel and other early observers stated that the affinities of the violet fungus were with the truffles. Persoon, Fries, and others placed the genus near *Sclerotium.* Tulasne considered the small sclerotia as probably a stage in the development of an ascomycete (pyrenomycete). This suggestion of Tulasne has apparently influenced many mycologists, and a search in this direction for the perfect stage has continued practically until the present time. Fuckel suggested that *Lanosa nivalis* Fr. might be considered the first or conidial stage of this fungus and he believed that the minute sclerotia or penetration cushions gave rise during the latter part of the season to pycnidia. With the more complete disintegration of the affected tissues he reported the development of a perithecial stage, and this fungus he called *Byssothecium circinans* (*Leptosphaeria circinans* (Fckl.) Sacc., *Trematosphaeria circinans* (Fckl.) Wint.). It will be noted that Winter regarded this

view of the genetic relation to *Rhizoctonia* as improbable; and Saccardo, who at first accepted the relationship, subsequently changed his opinion. Prunet ('93) states that he made certain inoculation experiments from which he was convinced that Fuckel was correct; but we possess no indications as to how these experiments were conducted. The writer in 1899, at Leipzig, germinated the spores of *Leptosphaeria circinans* and obtained a mycelium bearing no resemblance to the Rhizoctonia hyphae. The idea that *Leptosphaeria* constitutes a perfect stage of the *Rhizoctonia* has had no support recently, although Comes ('91) incorporates it in an extreme form in his treatment of the genus.

Rostrup ('86) found in the spring on the old roots of affected plants a pycnidial stage which he considered to be connected with the Rhizoctonia hyphae; and on the old roots of *Ligustrum* he found reddish filaments and scattering perithecia; the latter he identified as a species of *Trichosphaeria*. His assumption, however, has received no encouragement. When Hartig ('80) discovered a *Rosellinia* as the perfect stage of his *Rhizoctonia Quercina* there was a temporary revival of interest in the quest for one of the *Ascomycetes* as the perfect stage of *R. Crocorum*.

Frank ('97) reported observing the violet fungus on the grape, and associated with it he found a species of the *Thelephoraceae*. This he regarded as the perfect stage, and to the fungus he applied the name *Thelephora Rhizoctoniae*. This observation has failed of confirmation.

Eriksson ('13) has recently presented an extension of his earlier account ('03[a]) of diseases produced by *Rhizoctonia*, and in this he records a new "*Hypochnus*," *H. violaceus* (Tul.) Eriks. as the perfect stage of "*Rhizoctonia violacea*, Tul." In this he was stimulated by the observations of Rolfs ('03) and others in America, and Pethybridge ('11) in Ireland, on the occurrence of the basidial stage (*Corticium vagum* B. & C. or *Hypochnus Solani* Prill. & Del.) of *Rhizoctonia Solani* Kühn, resulting in a reëxamination of some material of the violet fungus on roots and stems of certain wild plants. This material had been preserved in alcohol thirteen

years earlier. The result of his study is reported as follows:

> "D'après ces renseignements, il faut—du moins pour ce qui concerne les formes du champignon qui envahissent les Carrottes—considérer comme résolue la question tant débattue de savoir à quel groupe rapporter le mycélium stérile connu sous le nom de *Rhizoctonia violacea.* Dans ce qui suit, je vais indiquer le nom scientifique qu'il faut donner, ainsi que les caractères diagnostiques du champignon autant que j'aie pu en juger sur les documents conservés que j'avais à ma disposition."

On the basis of these observations he creates the *Hypochnus* mentioned. No adequate diagnosis is given, but the important part of the account is as follows:

> "Ensuite le champignon forme autour des tiges de la même plante ou d'autres espèces de plantes immèdiatement au-dessus du sol, une enveloppe annulaire, membraneuse, d'un rose tendre, qui, montant souvent sur les tiges jusquà une hauteur de 5 à 15 mm. et s'étalant parfois sur la surface du sol comme une feuille toute mince, produit des basidiospores. C'est le stade *Hypochnus.*"

This apparently refers to material on *Stellaria media, Myosotis arvensis, Galeopsis Tetrahit, Erysimum cheiranthoides, Urtica dioica,* and *Sonchus arvensis,* which hosts he would regard as harboring the Hypochnus stage of that form of the violet fungus attacking the carrot, and for this reason the names just given appear in the list of hosts.

In the writer's opinion he properly considers it remarkable that the fructification stage should attack hosts other than those producing the sterile stage. In view of the character of the material, the incompleteness of the account, and the possibility of confusion with *Corticium vagum* B. & C. it would appear necessary to await confirmation of the observation that a *Corticium* (*Hypochnus*) may represent the perfect stage of the fungus here discussed, although, reasoning from the apparent relationship of this species to *R. Solani,* a Corticium stage might well be assumed. The writer has been unable thus far to secure any of the material mentioned.

In a footnote Eriksson expresses himself thus: "Quant à la Rhizoctone de la Luzerne, je suis porté à croire, d'après les

observations de cette année (1912), quelle doit être rapportée à un groupe d'Ascomycètes." This suggestion is both interesting and surprising since Eriksson adopts the Tulasnes' name for the *Rhizoctonia* on carrot and this would seem to concede the identity of the carrot and alfalfa forms. It is also in a measure inconsistent with his inoculation results, as reported later.[1]

CROSS INOCULATION AND CULTURAL STUDIES

The amount of cross inoculation work yet reported is not considerable, and for this, doubtless, the inability to cultivate the organism is largely responsible. Throughout the early literature numerous indications are offered showing that following a severe outbreak of the disease on any crop, it may appear on susceptible plants grown in the affected area—observations which tend to establish the identity of the fungus on different hosts. Among later observations may be mentioned those of Güntz ('99) who records that in a field where alfalfa and red clover had been seriously affected, beans, potatoes, and tuberous artichokes were planted; the potatoes subsequently developed the disease in serious form, and the other plants showed indications of its presence. In England it is reported (Bd. of Agr., '06) that potatoes are affected by the violet felt fungus, especially when following alfalfa; and under similar conditions the fungus appears upon clover, carrots, beets, and mangolds.

Eriksson ('13) undertook some cross inoculation work employing, in zinc cylinders, soil from diseased carrot fields (eight cylinders) in contrast with soil taken from areas free from the disease (two cylinders). At the same time, to the diseased soil he added pieces of carrots affected by the fungus. The cylinders were permitted to stand over winter

[1] Since obtaining proof of this paper I have received from Prof. Eriksson an advance reprint of his paper, "Fortgesetze Studien über Rhizoctonia violacea DC." Arkiv för Bot. 14 (Art 12) : 1 31. *f. 1-13.* 1915. It is impracticable to include here a full discussion of this paper. It is necessary to state, however, that he treats at length *Rhizoctonia Medicaginis* DC. and *R. Asparagi* Fckl., and includes inoculation experiments indicating form differences. After germinating the spores of *Leptosphaeria circinans* he comes to the conclusion that, in spite of his earlier work on *Hypochnus violaceus*, the pyrenomycete mentioned is the perfect stage of *R. Medicaginis.* Prof. Eriksson has also furnished material of *R. Asparagi* and of the *Leptosphaeria.*

and the following spring were planted to several varieties of carrots, to beets, mangolds, red clover, and alfalfa. At the time of harvest, the carrots were all more or less severely affected, while the sugar beets and alfalfa showed very light attacks, and the clover none at all. Continuing the work in subsequent seasons he obtained evidence in one case—that of the sugar beet—pointing to an increased virulence of the fungus with adjustment to that host. On the contrary, in the second year the alfalfa exhibited greater resistance, thus rendering a decision as to the existence of physiological races hazardous. He also reported, that on placing diseased soil and diseased carrots in a box in which various weeds were permitted to grow, the fungus appeared on eight species of weeds (representing several families), apparently a considerable proportion of those present. This also would seem to discourage the idea of marked host specialization.

Attempts to cultivate the violet fungus on artificial media have been made by several investigators without success. While in Leipzig, 1900, I obtained particularly good material on alfalfa from Bavaria. Dilution cultures were attempted both on various kinds of agar and on gelatin, but no growth of the fungus was secured in any case. Further trials were made with material from France in 1902, and again upon receiving comparatively fresh material from Kansas in 1911. Bailey ('15) reports an endeavor to cultivate the organism in Oregon, also without success. It is quite possible that special conditions are essential to its growth in artificial culture, but we should not assume that it is incapable of growth in this way. It would appear that the presence of contaminating organisms is not the sole cause of the difficulty, since isolated hyphae in the dilution cultures remain free from the growth of contaminating organisms, and yet themselves fail to develop a colony of growth. It will be recalled that Atkinson[1] found difficulty, but ultimate success, in growing *Ozonium omnivorum* (Lk.) Shear, the cause of the southwestern root rot of cotton. The writer also found that this organism is not readily cultured, but obtained a satisfactory

[1] Bot. Gaz. **18**: 16–19. 1893.

growth on cotton decoction starch paste in 1902. Since in general pathology and physiology the cotton *Ozonium* and the violet *Rhizoctonia* have much in common, a further careful investigation of their life relations would doubtless yield interesting results.

PREVENTION AND CONTROL

Relief measures respecting the violet fungus are very largely limited to the practices of good culture, good drainage, and sanitation. The early pathologists have generally recommended pulling up diseased plants and burning them. It is well to point out, however, that after a careful examination of the distribution of the fungus on the smallest fibrous roots, it has been found to invest these to a considerable depth in the case of alfalfa, and therefore a very small measure of security may be expected unless one carries out this recommendation in a far more thorough manner than is practicable in the field. The further suggestion has been made that where the diseased areas are few, small, and clearly defined, trenches may be dug to prevent the further spread of the disease; but if this should prove feasible under any conditions, it would be advisable only in connection with a thorough disinfection of the isolated areas by formaldehyde or sulphuric acid—the former disappearing from soil in time, and the latter being easily neutralized by liming. The rotation of crops is undoubtedly desirable, but complete immunity from the disease cannot be expected if we may trust the statements of Du Hamel and other observers to the effect that the fungus may remain alive in the soil for periods of from three to twenty years. The fact that many hosts are affected also complicates the practice of rotation.

The Common Rhizoctonia, R. Solani Kühn (Corticium vagum B. & C.)

EARLY STAGES

In addition to his discussion of the violet *Rhizoctonia* on beets and carrots Kühn ('58) described a disease of potatoes, of which the causal organism was recognized as a species of

Rhizoctonia differing notably from the violet organism, and to this potato fungus he gave the name *R. Solani.* The life history of the fungus and the symptoms of the disease induced were very imperfectly known at the time, so that the description could not be complete. As a result, those who subsequently discussed the genus *Rhizoctonia* have sometimes recognized *R. Solani,* while others have referred the organism to *R. Crocorum* (*R. violacea*), and still others have assumed that *R. Solani* Kühn was also the cause of another disease of beets and of carrots mentioned by Kühn without identifying the causal organisms. After a study of certain diseases in America induced by *Rhizoctonia,* I was keenly aware of this confusion, so when opportunity presented itself in the winter of 1899–1900 I conferred with Professor Kühn regarding those diseases, and also endeavored to obtain satisfactory specimens of the fungi. There has been no earlier opportunity to utilize the information obtained in connection with a general discussion of the genus.

Kühn laid special stress upon a scab ("Schorf oder Grind," later termed "Pockenkrankheit") of potatoes, sometimes followed by deeper seated injuries and decomposition ("als Räude und Krätze bezeichnet"). The symptoms are clearly those that we now know as one type (cf. McAlpine, '12) of the potato diseases ascribed to *R. Solani* Kühn (*Corticium vagum* B. & C.). It has been noted that the fungus was not so well described as might be wished, and the spores mentioned were evidently those of contaminating organisms, or else the oval cells of the tufted stage of the fungus; but when we use in connection with this general description Kühn's comparison of this plant with the violet fungus (Kühn, '58, p. 248) it is convincing that the fungus on the potato which he had under consideration was not *Rhizoctonia Crocorum.*

The sclerotia were also inadequately described and figured. With reference to that point, however, Professor Kühn stated that while a common form of the fungus on the tubers consisted of irregular superficial sclerotia, this form did not lead to serious consequences and therefore received less attention from him. Material of this superficial sclerotial stage was

furnished the writer by Professor Sorauer in 1900 (for a photograph see Duggar, '09, p. 477, fig. 219), and, subsequently, from other points in Germany. It is clearly the "black speck" form of the disease now generally recognized. Professor Kühn also identified cultures of the American fungus on sugar beets (Duggar, '99) as very close to, if not identical with, his *R. Solani.* In 1858 Kühn was obviously unaware of the fact that the violet fungus also occurs on potato in Germany; and, in fact, he told me in 1900 that it was subsequent to 1858 when he first collected specimens of the violet fungus on this host. "The violet fungus produces no serious epidemics of the potato in Germany," he declared. Professor Kühn was unable to locate type material of *R. Solani,* and such material is doubtless unavailable. Before presenting still other indications pointing unmistakably to their identity, I shall proceed on the basis that it is correct to refer the sterile stages of the commoner American *Rhizoctonia* on potato and other plants to *R. Solani* Kühn, and once studied comparatively there can be no confusion of this plant with *R. Crocorum* (Pers.) DC.

A disease of carrots was also described by Kühn with which no fungus was positively associated. The indications are insufficient to determine whether this was a fungus or a bacterial disease. So far as the writer is aware no disease of carrots in Europe due to *R. Solani* has since been reported, though in 1900 Professor Kühn stated as his opinion that carrots as well as beets in Germany were affected by a fungus similar to *R. Solani.*

The violet root felt fungus was clearly distinguished by Kühn ('58, see pp. 235–237, 243–249) in its occurrence on both beets and carrots. It is not possible to mistake his statements in which the organism on these hosts is referred to *Rhizoctonia Medicaginis* DC." Moreover, he nowhere suggests the combinations *R. Daucі* Kühn and *R. Betae* Kühn, which later crept into the literature of the subject. This fact makes it difficult to understand the nomenclature employed by Eidam ('87) and Comes ('91). In discussing a beet disease prevalent in Germany, Eidam refers the organism to *Rhizoctonia Betae* Kühn. He gives a description of the disease and of the fun-

gus, including its growth on culture media. It is clearly the beet disease now well known in America, and of which the causal fungus is referred to *R. Solani.*

Kühn did describe the symptoms of another disease of beets, and this last bears every indication of being the heart rot later known to be due to *Phoma Betae* (*Phyllosticta tabifica*), much discussed by Frank and others. Kühn's discussion of this other beet disease has been interpreted, also, in the way I have indicated by Prillieux and Delacroix ('91) and others outside of Germany. In my conference with him, Professor Kühn stated that the only Rhizoctonia diseases of beets and carrots which he knew in the vicinity of Halle in 1858 and earlier were those due to the violet fungus, and of these he exhibited specimens having the usual characteristics. From the evidence at hand, therefore, the Rhizoctonia disease of beets described by Eidam was new on that host. It would seem, then, that Eidam is the authority for the combination *R. Betae,* which he attributes to Kühn. In any case it becomes a synonym of *R. Solani* Kühn (*Corticium vagum* B. & C.).

In discussing the Rhizoctonia disease of potatoes in Europe Sorauer ('86) describes unmistakably the "black speck" or sclerotial form of the fungus, and while he, like many others, assumed that it would be found to belong among the *Ascomycetes,* it is obvious that the characteristics of this stage of Kühn's fungus were well recognized.

Among the forms of *Rhizoctonia* which he enumerated and discussed Comes ('91) includes *R. Dauci* Kühn, and *R. Betae* Kühn. In his discussion of the first-named he reviews Kühn's account of the violet fungus on carrots, already mentioned; but in the account of *R. Betae* Kühn he evidently refers both to Kühn's account of the heart rot of beets and to the Rhizoctonia disease of this host described by Eidam. Pammel ('91) was the first American pathologist to report in this country a disease now known to be caused by *R. Solani.* He, however, followed Comes and Eidam in referring to the fungus causing the beet rot as *R. Betae* Kühn.

Atkinson ('92, '95) studied a "sterile" fungus causing sore

shin or damping off in cotton, and ascertained that the same fungus was commonly associated with, and capable of, inducing damping off of various seedlings in the greenhouse.

Duggar ('99) also referred to the beet rot fungus in America as *Rhizoctonia Betae* Kühn, following Comes, and was able to determine that this beet fungus was identical morphologically (mycelium and sclerotia) with the damping off fungus found by Atkinson. The characteristics of the two organisms in culture were also identical, both forming on certain media a rich mycelium and finally numerous flaky or tufted centers of growth, some of which become irregular, often crust-like, sclerotia. Neither on affected seedlings nor on beets were sclerotia ordinarily produced (compare, however, Edson, '15, pl. 23).

Subsequently, Duggar and Stewart ('01) reported that several types of disease, on a variety of hosts, including the potato, were induced by *Rhizoctonia*. The account given was intended to be merely preliminary, and for this reason a few words of explanation are necessary. The account referred to did not (perhaps unfortunately) explicitly indicate that, as far as the studies had progressed, there was evidence that the organism, or forms of the organism (except in the case of the form on rhubarb, referred to later) exhibited morphologically and in culture the characters of the beet rot and damping off fungus. The authors were likewise convinced, after a study of European material of Kühn's fungus on the potato, of the identity of the American and European forms on this host. Cultural studies were being carried forward with *Rhizoctonia Solani* from many hosts, since there was the possibility of establishing definite forms or races, of finding the perfect stage, and of discovering other species. Again, specimens of the violet root felt fungus on various hosts had been obtained by one of us, and it was intended to include in a final paper a general account of the genus.

This failure to designate the form with which we worked has doubtless led to some misunderstanding (see Prillieux '97, Eriksson '13, p. 17). However, in a more recent account (Duggar, '09, pp. 477–478), it will be seen that the diseases

discussed are ascribed to *R. Solani* (*Corticium vagum* B. & C.).

DISTRIBUTION

Rhizoctonia Solani is distributed throughout the United States and Canada. There is every reason to believe that it exists as a saprophyte in most arable soils, and under certain conditions may attack many species of plants. It is perhaps most frequently noted as a damping off disease in greenhouses and seed beds, but this occurrence may be explained by the fact that here the conditions are probably more conducive to the pathogenicity of the fungus. On the potato it is likewise wide-spread, although, as noted later, the economic importance of the diseases induced varies in different sections of the country, probably in accordance with climatic and soil conditions. In all potato-producing states and regions it is a well-known disease. On the sugar beet it has been observed in many states. The fact that it is an important disease of one crop or another in every section of the country is alone sufficient indication of its general occurrence. *Rhizoctonia* has been mentioned in Brazil by Potel ('00), but it is not clear to which species he refers.

It is rather surprising to find that *R. Solani* has received relatively little attention in Europe. Although recognized as inducing a disease of the potato widely distributed in central Europe, and occasionally reported on the beet, yet little careful work has been bestowed upon the fungus. Eriksson ('13), seems to be unfamiliar with the fungus in Sweden. On this account we can gain no incidental information regarding *R. Solani* as a result of his extensive studies of the related species in that country. The following will express his attitude regarding *R. Solani:*

> "Il paraît très douteux, du moins si l'on en juge d'après les descriptions et les figures données, que les nouvelles formes de la Rhizoctone stérile signalées dans ces derniers temps par B. M. Duggar et F. C. Stewart sur une quantité de plantes différentes en Amérique (* * *) soient vraiment identiques aux formes du *Rhizoctonia violacea* qui ravage l'Europe."

We have very little data regarding its occurrence in other sections of continental Europe, although from conference

with Prof. Delacroix in Paris (Nov. 28, 1901) and from an examination of material furnished by him I learned that it is not uncommon throughout France on the potato. It will be recalled that the perfect stage was described by Prillieux and Delacroix ('91). Judging from the amount of the black speck disease observed on the potato in the markets of various cities in southern Europe during 1905–'06 the writer would infer that it is of more frequent occurrence than is reported. Pethybridge ('11) finds the fungus (including the Corticium stage) well distributed in Ireland, and it is reported from other parts of Great Britain.

McAlpine ('11) has reported this fungus on the potato from several points in Australia, and he states that it occurs upon a variety of economic plants. Since it has proved a serious disease in very few localities, it receives little attention, and is therefore freely disseminated by commercial intercourse. It is also known in New Zealand and Japan.

The investigations of Shaw ('13) suggest that *Rhizoctonia Solani* may be an important disease-inducing organism in some of the more humid regions of India. Reference is made later (pp. 448–450) to the fact that he has obviously misapplied this name, however, and also that other confusion has resulted. In spite of this, it seems certain that he has observed all stages of the fungus.

TYPES OF DISEASES INDUCED, SYMPTOMS

It is not my purpose to attempt a complete description of the more important diseases caused by this species, yet sufficient will be included to indicate the main types of diseases thus far investigated, their general distribution, and their striking pathological relations. By types of disease, I have reference to general effects or symptoms. The effect of the fungus upon the stems may occasion a different appearance from its action upon the root, and thus there arise the different types referred to. With respect to penetration and action upon the cell the behavior of the fungus may be the same in all cases. Moreover, as a result of the primary injury, secondary effects may occur, and sometimes such secondary phe-

nomena may be so striking in appearance as to dominate the primary injuries or lesions.

For convenience we may arrange the types of disease in the following categories: (1) damping off, (2) stem rot, (3) root rot, (4) leaf rot, (5) scab, and (6) such secondary effects as rosette, little potato, and leaf roll. Since more than one type of disease may occur upon a single host, and especially since one form of the disease may grade into another, it will be more practicable to discuss these under the following captions: (1) damping off, (2) potato diseases, (3) rot of fleshy roots, (4) stem and root rots of herbaceous plants, and (5) fruit and leaf injuries.

DAMPING OFF

It would appear that the first mention of a disease of seedlings caused by *Rhizoctonia* is that of beets, recorded by Eidam ('87), although he gives no complete account of the evidence. It is preferable to date our knowledge of damping off diseases caused by *Rhizoctonia* from the work of Atkinson ('92), who studied particularly sore shin of cotton, but he also found the "sterile" fungus to cause damping off of seedling beets, radish, lettuce, egg plants, cabbage, and other plants in the forcing house. The later identification of the fungus concerned (Duggar, '99) and its association with the damping off of various plants (Duggar and Stewart, '01) was only the beginning of the observations which have now served to direct our attention to the vast importance of this fungous disease throughout the United States both in the greenhouse and in the outside seed bed.

Among numerous instances in which damping off has been reported due (or in all probability due) to this fungus may be noted the following: (1). It has been found as a source of serious injury to ginseng in the seed bed (Van Hook, '04; Whetzel and Rosenbaum, '12). (2). Tobacco seedlings are so frequently injured that soil treatment has received special consideration in the case of this crop (Selby, '04; Cook and Horne, '05). (3). As a damping off disease of cotton (sore shin) it occurs not only in America but in Africa (Balls, '05, '06) and possibly in India (Shaw, '13) as well. (4). Tomato

seedlings seldom attacked by *Pythium* have been found to succumb to *Rhizoctonia* in Louisiana (Edgerton and Moreland, '13). (5). Alfalfa seedlings have been reported susceptible in one instance (Stewart, French, and Wilson, '08). (6). Seedlings of various species of conifers from a few days to nine weeks old have been reported attacked in several instances (Hartley, '12, Clinton, '13).

The majority of the instances reported above were under normal seed bed or field conditions. Many other cases of the damping off of seedlings might be included where seeds are grown in crowded condition in moist greenhouses. Again, damping off of cuttings by *Rhizoctonia* is now a well-known phenomenon in the propagating house, and special precautions are taken with respect to drainage and moisture in order to reduce the injuries to a minimum. It is safe to assume—since the fungus seems to be found in practically all soils—that it is in general the worst enemy of seedling plants. In fact, it may be anticipated that under conditions favorable for the fungus the damping off of seedlings of numerous species may be anticipated. So far as the writer has been able to ascertain there has been no report of the damping off of monocotyledonous plants under normal seed bed conditions.

While *Rhizoctonia Solani* may perhaps induce damping off in innumerable species regarding which observations are lacking, some of the host plants which have come to the writer's attention as particularly susceptible are the following: lettuce (*Lactuca sativa*), celery (*Apium graveolens*), beet (*Beta vulgaris*), cress (*Lepidium sativum*), tobacco (*Nicotiana Tabacum*), balsam (*Impatiens balsamina*), snapdragon (*Antirrhinum majus*), cotton (*Gossypium* spp.), cucumber (*Cucumis sativus*), squash (*Cucurbita* spp.), sunflower (*Helianthus annuus*), carrot (*Daucus Carota*), radish (*Raphanus sativus*), and phlox (*Phlox Drummondii*).

Since the phycomycetous damping off fungus *Pythium* has been known to pathologists much longer, and prior to 1895 was practically the only fungus to which this type of disease was ascribed, it is probable that much damage due to *Rhizoctonia* has been ascribed to *Pythium*. Moreover, unless

examined microscopically, there are no symptomatic differences between the effects of the two organisms.

Seedlings affected exhibit symptoms somewhat different with age. The youngest seedlings of all delicate plants show what may be called the usual damping off characteristics. Near the base of the stem an hygrophorus or translucent appearance is quickly followed by shrinkage of the tissues and weakness of the stem. The plants topple over, the fungus invades all parts, and spreads rapidly to the neighboring individuals. The cells of the sap-perfused tissues are flaccid and injured, some showing this even before the entrance of the hyphae into the cells. Somewhat older plants and the more robust seedlings of cotton, bean, etc., often exhibit characteristic lesions. Atkinson ('95) gives a description of its effect on cotton seedlings as follows:

> "The trouble is caused by the fungus growing first in the superficial tissues of the stem near the ground and disintegrating them before it passes to the deeper tissues; in other words the fungus never seems to penetrate far in the living tissues, but 'kills as it goes,' and the tissues become brown, depressed, and present the appearance of the plant having a deep and ugly ulcer at the surface of the ground. The fungus does not spread into the tissues either above or below the ulcer to any extent, but literally eats away at that point until it has severed the stem at the affected place or the plant has recovered from its effects."

DISEASES OF POTATOES

The potato is the most interesting of the host plants with respect to the parasitism of *Rhizoctonia* by reason of the many types of disease induced under diverse conditions. The conditions may be in part climatic and, in part perhaps, dependent upon the pathogenicity of the particular strain of the fungus or upon the stage and development of the host at the time of infection. It has been noted that when Kühn first described the disease of potatoes in Germany he laid emphasis upon a scab which was often followed or accompanied by decay. This form of the disease was probably less prevalent in the country as a whole at that time, and the more recent accounts indicate that the "black speck scab" or "black speck," properly the sclerotial stage, is the feature by which the main type

of the disease is now generally known. At present the following main types of injury are recognized for the potato: (1) black speck scab or sclerotial stage, (2) Rhizoctonia scab, (3) Rhizoctonia rot, (4) stem lesions and root rot, (5) rosette and leaf roll, and (6) little potato and aerial potato.

Black speck is a form of the disease most widely distributed and in itself scarcely merits consideration as a "disease" at all, since the sclerotia are superficial on the tuber, and it is merely the appearance of the potato which is affected. The sclerotia may lead to other types of disease which are more serious. The black specks show up most clearly when the potatoes are wet and it is only at this time that they present the appearance of being black, for, as indicated later, the normal color of the sclerotia is deep brown. It was this form of the disease which first gave evidence of the wide distribution of the fungus in America (Duggar and Stewart, '01), and it has been shown to exist in practically all potato-producing sections of the United States and Canada. It occurs throughout Europe, especially on the later varieties of potatoes. It is also reported from India, Africa, and Australia, so that it may be assumed to be world-wide in its distribution on this host. It is safe to say that this is the only form of the disease which does not result directly in serious injury and loss to the crop. In the United States, especially from Ohio westward, other forms of the potato disease assume a seriousness nowhere else attained. If all such forms of the disease mentioned below occur in the Atlantic states they are of little consequence. They are, moreover, far less frequent in Europe, India, and Australia.

The Rhizoctonia scab is believed to occur as a result of the penetration of hyphae during the early stages of sclerotial development, and occasionally it may be induced by a late growth of new hyphae from old sclerotia. The writer has had an opportunity of examining only casually this form of the disease. It is one of the types doubtless seen by Kühn. According to McAlpine ('11), when this disease occurs, practically every part of the tuber is affected, no normal skin remaining. In severe cases the scab areas may be thrown into folds or puckers and these rub off easily in the form of "cork dust."

It is reported that the irritating hyphae are then found at the bases of such scab formations. This scab has been reported fairly common in Europe and in Australia. Güssow ('05) seems to refer to the same type in England, and Rolfs ('03) describes it from Colorado. Specific scabs of the potato have been clearly defined and related to particular organisms. The capacity of the tuber to respond with cork formation to varied injuries suggests that in certain modifications of Rhizoctonia scab this fungus may accompany other active scab inducing agents.

The Rhizoctonia rot is a form of disease which appears relatively late in the season when certain conditions prevail, or possibly when the fungus has for one reason or another developed unusual virulence. The disease is supposed to originate either from stem infections, from sclerotia, or from scab areas. In any case penetration of the mycelium occurs to a considerable depth, and according to McAlpine ('11) there is produced in Tasmania a form of the disease known as brown rust, characterized in the early stages by dark spots in the tuber resembling certain symptoms of *Phytophthora.* It may also be associated more or less with the deeper form of the Rhizoctonia scab. During the latter part of the season a typical stem rot may occur which is not characterized by the definite lesions described later. Instead, the affected cortex slips readily from the wood and about the bark a considerable web of the yellow-brown hyphae may be found superficially, below and just at the surface of the ground, and the pith may be fairly stuffed with the mycelium. Plants only slightly affected with this form of the disease, especially when growing on rich garden or muck soil, have been found to yield the collar or *Corticium* stage.

It is not always easy to distinguish as separate forms of the disease, stem lesions, rosette, little potato, aerial potato, rolling, etc., for these types of injury are often associated. All of these types except stem lesions are properly secondary effects, and there is abundant evidence that all represent responses of the plant to disturbed condition or nutrition, sometimes associated with native weakness. It would not be

strange, therefore, if somewhat similar effects should characterize, as they do, purely "physiological" disturbances. Stem lesions are generally dark, sunken areas, clearly different from black leg, occurring at the surface of the ground or on any of the underground stems, or tuber-forming stolons. These lesions may result in the early death of the affected plants. Selby ('02, '03) maintains that generally the lesions upon young shoots are associated with stunted growth and the production of rosette-like clusters of the upper leaves, as well as with less marked modifications of habit, including slight leaf rolling. Drayton ('15) finds the hyphae in the lesions.

If the tuber-bearing stolons are the seat of injury, the food supply is cut off from the young tubers and there may result "little potato," a form of the disease which Rolfs ('04) has found to be an important cause of the potato failures in Colorado. Little potato in Australia is considered an evidence of underground injuries occurring late in the season. Injuries which effectually girdle the stem, especially if these occur during a moist season or when the crop is frequently irrigated, lead to the formation of aerial tubers. In the relation of *Rhizoctonia* to the various types of potato diseases much remains to be investigated, and Orton ('14) rightly suggests that inadequate attention has been bestowed upon the question of the predisposition of the tubers used as seed, since it is quite possible that these may yield offspring with tendencies toward rosetting, leaf rolling, and other morphological modifications.

ROT OF FLESHY ROOTS

The root rot of beet, apparently first described by Eidam ('87) in Germany, and shortly afterward found by Pammel ('91) in Iowa, was observed in New York (Duggar, '99) some years later. Since that time it has appeared epidemically in Nebraska (Lyon and Wianco, '02) and other western states. The fungus is most virulent during midsummer or later. Infection may take place at the bases of the leaves or on the fleshy root. The leaf bases blacken, the leaves become paler, and finally wilt. Pammel ('91) has drawn attention to the

fact that when fleshy root crops of this type are attacked by such fungi they die gradually, while herbaceous plants (cotton, alfalfa, etc.) wilt suddenly. This is probably closely related to the effect of the fungus on the conducting tissues. In the beet root the invaded tissues are pale brown, and often cracks or rifts occur, though rotting may take place without such lesions. Sometimes there is partial recovery after the cracks are formed, and in this case callous tissue is developed.

A soft crown rot of the radish induced by this fungus has apparently been reported only once (Duggar and Stewart, '01). A similar disease of the carrot was found in 1900 in New York and this is possibly the disease first reported by Kühn ('58, pp. 241–243), although he did not identify it as due to a *Rhizoctonia*.

STEM AND ROOT ROTS OF HERBACEOUS PLANTS

Rhizoctonia Solani produces serious stem and root rots of a number of economic herbaceous plants, among which the following are known to be important: carnation (*Dianthus caryophyllus*), Sweet William (*Dianthus barbatus*), bean (*Phaseolus vulgaris*), sweet-pea (*Lathyrus odoratus*), and violet (*Viola odorata*).

The carnation stem rot is one of the most destructive diseases occurring on this host and is wide-spread in the United States. The general symptoms of the disease on carnation and Sweet William are much the same. The stem is affected at or just below the soil level. The fungus penetrates and kills the cortex which may be readily slipped from the wood. Through the medullary rays the hyphae also enter the pith, which likewise decays. In later stages of the disease the wood shreds, due to the complete penetration by the fungus of all parenchymatic tissues.

Several important epidemics of *Rhizoctonia* on bean have been reported from different parts of the United States. In addition to the outbreak described by Duggar and Stewart ('01), Hedgcock ('04), a few years later, found the bean disease severe near St. Louis. The base of the stem and the larger roots bore characteristic ulcerations; pods were af-

fected, and through the sunken areas of these the hyphae penetrated the seed and produced small sclerotia on the seed-coats. The fungus was cultivated and typical Rhizoctonia hyphae and sclerotia were obtained. Fulton ('08) observed the disease in Louisiana on stems and pods, with the characteristic ulcerations, especially at the surface of the soil or just below. He proved the causal relation of the organism through cultures, and inoculations yielded positive results with the damping off of seedlings. McCready ('10) reported the bean disease as new to Ontario, where it was also characterized by stem and pod ulcers. In New York Barrus ('10) observed an epidemic of this host in which as many as 30 per cent of the plants were affected. He determined the fungus by cultural studies and proved its pathogenicity by inoculation. On the sweet-pea the disease is mainly a root rot, yet the base of the stem may also be considerably affected before the plant succumbs. On the violet it is primarily a crown disease, but where the plants are succulent and the conditions are moist, the leaves are considerably invaded.

FRUIT AND LEAF INJURIES

In discussing stem diseases the occurrence of *Rhizoctonia* on bean pods has been mentioned. Another case of fruit injury is described by Wolf ('14), who found a severe rot of egg plant fruits from which the fungus was obtained. The pathogenicity of the organism was determined by inoculations, and cross inoculation from tomato and potato led to the conviction that the organism was *Rhizoctonia Solani*.

Direct attacks of leaves by *Rhizoctonia Solani* are infrequent. From the habits of the fungus this would be expected. The one serious leaf disease reported is that of lettuce (Stone and Smith, '00), in which the fungus spreads over the whole surface, causing a moist rot. Sclerotia are frequently formed in connection with this affection. It would be anticipated, perhaps, that diseases of a similar nature might be found on other plants with the rosette habit. Leaf stalks are frequently invaded, or may be the regions of first attack, in the case of the beet disease. The disease of leaf stalks of rhubarb

reported by Duggar and Stewart ('01) is not due to typical *R. Solani.*

MYCELIUM AND SCLEROTIA

The morphological characteristics of the hyphae and sclerotia have been adequately described by several writers, but it may be well to summarize some of the more important features. Upon such hosts as the potato, sugar beet, carnation, and others there is more or less development of an external web, but never over the general root system such a complete investment of roots by a mantle of hyphae as characterizes the violet fungus. The external hyphae are somewhat colored, usually yellowish brown, and they are generally of two types. One type may be designated as purely vegetative and another as constituting the external tufts or masses when these occur. All hyphae are practically colorless when young, vacuolate, more or less irregular, septate with the septa at intervals of 100–200 μ. The diameter of vegetative hyphae is 8–12 μ. Branches arise, and when young these are inclined in the direction of growth and are invariably somewhat constricted at the point of union with the main hyphae (fig. 5). As the hyphae mature and become more deeply colored they are more uniform and rigid, the distances between cross walls are greater, the constrictions where branches arise less marked, and the branches are approximately at right angles to the main hypha.

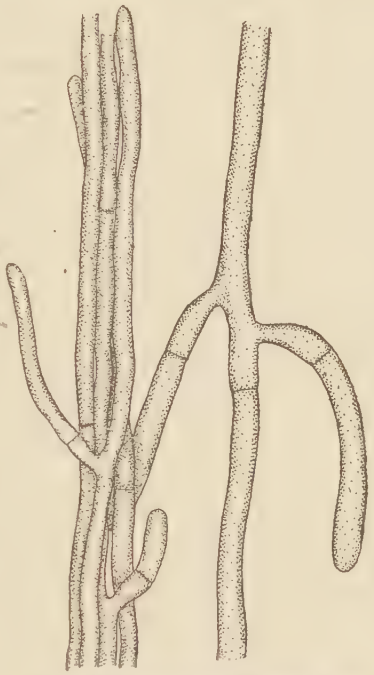

Fig. 5. *Rhizoctonia Solani* (*Corticium vagum*): A vegetative hypha and a small strand from artificial culture on potato.

On certain affected plants a short tufted or mealy growth occurs and this is made up of hyphae of very different characteristics. In the young condition threads are profusely

branched and lobed, sometimes botryoid, and they are ultimately divided into short, ovate cells, arranged in short chains, or elbowed, and producing branches in a more or less dichotomous fashion (figs. 7 and 8). In culture the denser

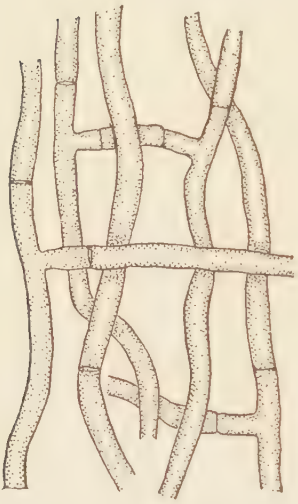

Fig. 6. *Rhizoctonia Solani:* Vegetative hyphae.

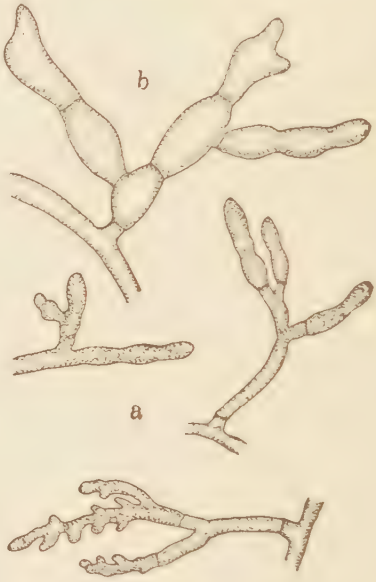

Fig. 7. *Rhizoctonia Solani:* a, young hyphae from young sclerotial tuft on lettuce; b, older cells from same source.

masses give rise to sclerotia. With maturity these hyphae become light brown in color, they break up readily into short hyphal lengths or single cells, the individuals of which bear some resemblance to conidia. However, they could not easily be mistaken for spores, although they may function as such, inasmuch as most of them may germinate within a few hours when placed under suitable conditions. I have previously described ('99) this process as follows:

> "So far as observed, germination is always by the protrusion of a tube through a septum. When several cells are connected, a germ tube from one cell may pass into and through its neighbor, * * * *, and thus peculiar appearances may result. Some of the cells of the hyphal chains seem to be devoid of protoplasm, and from neighboring protoplasmic cells the germ

tubes seem to pass into such empty cells as readily as directly into the nutrient solution. When the germ tube is from 10 μ to 20 μ in length, it is invariably narrowed towards the outlet from the parent cell, and a septum forms at a short distance from this outlet."

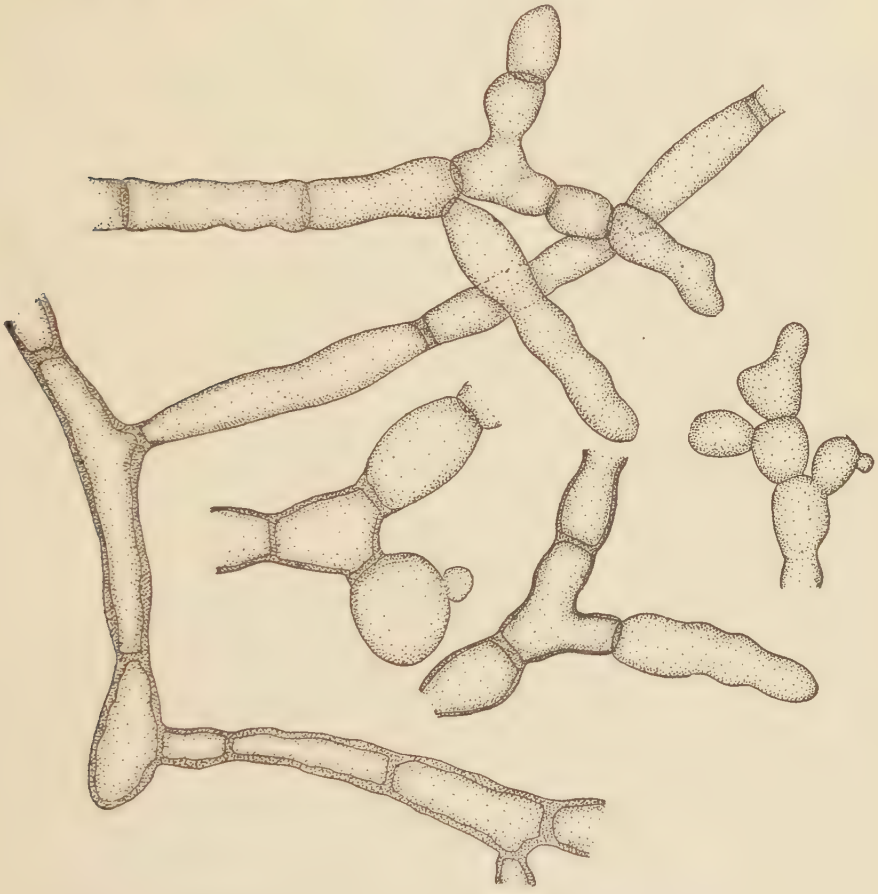

Fig. 8. *Rhizoctonia Solani:* Lobulate, moniliform, and elbowed cells from tufted growth in artificial culture.

The hyphae which penetrate the tissues remain colorless so long as they are in active growth, and while generally less in diameter they present much the same appearance as the young external hyphae. In the different strains which have been studied, originating from different hosts, certain minor modifications of the general habit of the fungus in culture have been observed. But these have not seemed to be suffi-

cient to be considered of specific importance, except in the case of the form on the rhubarb. In general, the differences referred to consist in a variable amount of the mealy or tufted growth, or of the amount of aerial growth; differences in the color of the colony are also observable; and the rapidity with which sclerotia are formed are all minor distinguishing features. The subject needs further investigation, but in general it is felt that these differences are such as might be due to permanent differences in the pathological strains, on the one hand, or may be regarded as temporary differences due to the recent environment, on the other. It may be pointed out that the appearance of the mycelium of the beet fungus from the damping off seedlings is not exactly comparable with that of the mycelium derived from the beet rot. When the organisms from both sources are grown in culture they are found to be identical. Strains do occur, however, evidence of which may persist for some time in the general appearance of the cultures.

The exact conditions under which sclerotia may occur on the various hosts affected have not been determined. It has been noted that affected potato tubers are the main seats of sclerotia formation when the fungus attacks that host. Upon this plant they are typical, and the numerous illustrations published are sufficient evidence that the appearance is much the same under a variety of conditions. Special attention may be called to the illustrations of Duggar and Stewart ('01), Rolfs ('02), Duggar ('09), McAlpine ('11), Pethybridge ('11), and Morse and Shapovalow ('14). On the majority of hosts, however, sclerotial formation is relatively rare.

From the various illustrations referred to it will be seen that the sclerotia vary in size from those so minute as to be scarcely visible, to others which may be a centimeter or two in diameter. They are generally more or less flattened, irregular, deep chestnut-brown, and generally smooth on the surface (that is, free from a looser growth of investing hyphae). Smoothness of sclerotia, which has been regarded by Kühn as of much diagnostic value, should not be considered

an important character except under natural conditions. Sclerotia which develop on fleshy organs in moist chambers as well as those which develop in culture show to a certain degree, a semi-persistent hyphal investment; but such investing hyphae are readily worn away, whereas in the violet fungus they are truly persistent.

Sections of the denser sclerotia exhibit a fairly homogeneous structure (fig. 9), with the cells more uniform in size and appearance than in *Rhizoctonia Crocorum.*

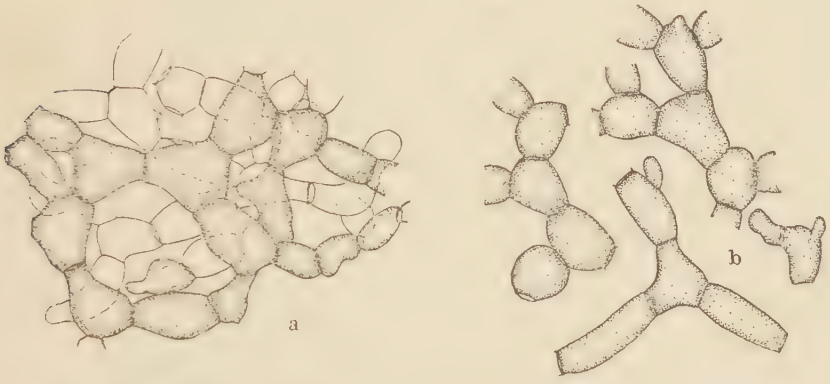

Fig. 9. *Rhizoctonia Solani:* a, from a section of sclerotium on potato; b, cells isolated by maceration of sclerotium.

THE BASIDIOSPORE STAGE, SYNONOMY, AND MATERIAL EXAMINED

Besides suggestions of a general nature no indications regarding the perfect stage of *Rhizoctonia Solani* were made prior to the discovery of the *Corticium.* Prillieux and Delacroix ('91) described *Hypochnus Solani* from potato stems, and although at this time the Rhizoctonia diseases were known in Europe no connection with this Hypochnus stage was suspected. The characteristic collar of mycelium was found surrounding the stem just above the surface of the ground, but they found nothing to indicate that the fungus had injured particularly the plant affected.

Rolfs ('03) found the collar fungus during his studies of potato diseases in Colorado. The material was determined by Prof. E. A. Burt as referable to the species *Corticium vagum* B. & C. On account of the parasitic habit, however,

it was considered advisable to make the fungus a variety of the Berkeley and Curtis species, so that it was written *Corticium vagum* B. & C. var. *Solani* Burt. Prof. Burt also recognized that it agreed closely with, and might be identical with, *Hypochnus Solani* Prill. & Del. This conclusion the writer accepts, but in view of the fact that Professor Burt is preparing a monograph of the *Thelephoraceae,* I shall not discuss this point; for the same reason I need only express doubt regarding the validity of Shaw's suggestion that *Hypochnus ochroleucus* Noack and *Corticium vagum* B. & C. are identical, although there is a certain similarity in the various stages.

Rolfs ('04) was able to germinate the basidiospores and to develop characteristic Rhizoctonia hyphae from these. Riehm ('11) also reported germinating the basidiospores and producing a characteristic Rhizoctonia mycelium together with the formation of sclerotia. Pethybridge ('15) gives a more complete account of mycelial production from spores.

The herbarium and fresh material which has been examined and found to agree with the authentic descriptions of *Rhizoctonia Solani* Kühn (*Corticium vagum* B. & C.) may be briefly enumerated:

Exsiccati: *Rhizoctonia Napaeae* nov. sp., Westendorp and Wallays, Herb. Crypt. Fasc. 5: 225. (On decaying turnips which had been stored in a cave.)

American material: Hyphal stages on numerous hosts, many of which are mentioned in this paper, also others not included; sclerotia, on potatoes grown throughout the eastern and central United States, on potato stems (New York, 1900), on bean pods (New York, 1910), also on carnation stems, lettuce leaves, etc. Corticium stage from Prof. F. H. Rolfs, Colorado, 1901, on potato stems; from Dr. I. C. Jagger, Rochester, New York, 1914, on potato stems and on crown of carrot; from herbarium of Prof. E. A Burt, material on moist soil and decayed wood, collected by Prof. Farlow, Magnolia, Mass., 1903; from Herb. Mo. Bot. Garden, Nos. 44679, 44681, and 44682; collected by Dr. Geo. L. Peltier, Urbana, Ill., 1915.

European material: Sclerotia on potato tubers from Prof.

Sorauer, Berlin, 1900; from Prof. Magnus, Berlin, 1901; from Prof. Delacroix, Paris, 1901; and material secured on the markets of various cities, 1905–06.

As far as the writer has been able to determine, the following synonomy may be listed for *Corticium vagum* B. & C.:

Rhizoctonia Solani Kühn (1858).
Rhizoctonia Betae Eidam [non Kühn] (1887).
Rhizoctonia Napaeae West. (1846).
Rhizoctonia Rapae West. (1852).
Hypochnus Solani Prill. & Del. (1891).

PREVENTION AND CONTROL

Much the same situation confronts us regarding the prevention and control of *Rhizoctonia Solani* as in the case of *R. Crocorum*. The presence of the fungus in practically all soils serves to emphasize the importance of cultural methods including drainage and sanitation. In this case, however, since the fungus is of so much importance in the seed bed and in the greenhouse special preventive measures may be practised. Selby ('06) found that the treatment of the seed bed with formalin (1:160 to 1:200) proved satisfactory in most cases. In general, the best results have been obtained by steam sterilization, and where the facilities are at hand it is practicable to apply this to any type of greenhouse work, and, in certain cases, to seed beds outside. Liming has been recommended for the control of the disease in the field, but this has not been uniformly successful, and cultural studies have shown that the fungus is able to withstand a high percentage of alkalinity. Nevertheless, when liming results in the improvement of physical and sanitary conditions of the soil it undoubtedly assists in restraining the activity of the fungus in an indirect way, possibly by raising the resistance of the host.

Even though the fungus may be widely distributed, it is advantageous to plant clean "seed." This applies particularly to the case of the potato. The presence of the sclerotia upon the tuber makes possible the early spread of the fungus

to the young shoots. It has been positively determined that the more effective tuber treatment is the standard corrosive sublimate solution, as for potato scab. In all cases, however, it would be better to employ seed which are not infected, if this is possible.

Conclusions and Notes

In the account already given of *Rhizoctonia Crocorum* perhaps sufficient discussion of the occurrence and the characteristics of this form has been entered upon, except in the way of a direct comparison between this species and *R. Solani*, subsequently included. Further work upon the first named species should consider especially the culture of this organism, inoculation experiments, the development of the organism as it occurs on several hosts, the formation of sclerotia and infection cushions, and the confirmation or more definite declination of Eriksson's view that the fungus is referable to *Corticium* (*Hypochnus*). From the study of this organism thus far the following conclusions seem justified:

1. The views of L. and C. Tulasne that the forms of *Rhizoctonia* on crocus, alfalfa, and other hosts may be included in a single morphological species is confirmed.

2. The correct name of the violet root felt fungus, so long as a spore stage remains uncertain, is *Rhizoctonia Crocorum* (Pers.) DC.

3. This organism occurs throughout a considerable part of Europe and has been found in a few localities in America.

4. It attacks a variety of plants representing many families, mostly dicotyledonous.

5. The mycelium and sclerotia exhibit no important differences in equivalent stages on the different hosts, but large sclerotia which form freely in contact with crocus, and often near the affected roots of alfalfa, are seldom observed in connection with the attacks upon beets, carrots, and some other hosts.

6. The existence of distinct forms or races of this species requires further extended study.

7. The organism has not yet proved culturable with the usual laboratory methods.

8. At the present time there is insufficient evidence to determine what the perfect stage of this organism may be.

Obviously much still remains to be done regarding the physiological, pathological, and taxonomic relationship of the culturable forms which in the vegetative stage may be referred to the form-genus *Rhizoctonia*. The writer has grown in culture *Rhizoctonia* from twenty-three different American hosts, most of which are mentioned by Duggar and Stewart ('01). Most of these were grown upon a variety of culture media including prune juice, beet, and potato agar; also beans, stems and pods, celery, sugar beet and potato cylinders, and corn meal mush. With one exception (the organism from rhubarb) the cultural characteristics have been sufficiently similar, especially after protracted culture in the laboratory, to suggest a single species, with characteristics of the beet and cotton fungus, already sufficiently described (Atkinson, '92, '95; Duggar, '99). Moreover, these cultural studies have confirmed in all cases the conclusions tentatively arrived at from the preliminary microscopic examination of the fungus on the different hosts. Reasons have already been given to indicate why this species is properly *R. Solani*. It is recognized, however, that much culture and inoculation work is necessary to establish the point that the fungus on the various hosts is the same species, and to determine to what extent physiological forms may occur.

The following brief summary of conclusions may be presented with regard to *Rhizoctonia Solani:*

1. The common American species of *Rhizoctonia* is *R. Solani* Kühn.

2. This fungus is widely distributed in America and elsewhere, and would seem to occur on the potato in most regions of the world where this crop is a staple product.

3. The host plants represent many families of dicotyledons, *Asparagus Sprengeri* being the only monocotyledonous host thus far reported.

4. The types of disease induced are most diverse, damping off and root and stem rots being the most important direct effects. Secondary effects have been studied only in a few localities.

5. The mycelium and the sclerotia, as well as the general appearance on the host, readily distinguish the fungus from *Rhizoctonia Crocorum* (Pers.) DC.

6. The organism is readily culturable by the usual laboratory methods.

7. The evidence seems clear that the perfect stage of this organism is *Corticium vagum* B. & C.

It is to be regretted that the fungus causing a disease of rhubarb (Duggar and Stewart, '01) was lost before adequate study could be bestowed upon it. The fungus bore a close resemblance to *Rhizoctonia,* but the aerial hyphal cells were shorter and of greater diameter than those of *R. Solani.* No sclerotia were found on the host, and they did not develop in culture.

Shaw ('13) has contributed interesting notes on diseases of plants in India attributed to two species of *Rhizoctonia.* Unfortunately, however, he has added to the general confusion regarding this subject by a preliminary discussion which does not sufficiently designate the forms referred to, but more especially by the advancement of certain ideas regarding species which are made, apparently, without adequate study of material from other countries. The conclusions arrived at are necessarily at variance with our present knowledge of the forms of *Rhizoctonia.*

Of the organisms producing diseases in Indian crops he refers to *Rhizoctonia Solani* Kühn, a fungus which he found on jute, mulberry, cotton, groundnut, and cowpea. The mode of branching of young hyphae of his fungus is characteristic of *R. Solani,* but with this the resemblance apparently ceases. Basing an opinion wholly upon his descriptions and figures, the adult mycelium (Shaw, '13, pl. 7 and 8) differs from *R. Solani* (1) in being usually much finer; (2) in the abundant development of short "barrel-shaped" cells in the ordinary

vegetative mycelium, which would seem, from his figures, to have little in common with the chain-like, ovoidal, often branched or lobed cells (designated "barrel-shaped" by Balls) of *R. Solani* (see Atkinson, '92, '95; Balls, '05, '06; Duggar, '99; Duggar and Stewart, '01; and others); and (3) in the verrucose or warty, wall markings (Shaw, '13, pl. 8, figs. 2–3), all of which indicate some other fungus.

Again, the development of sclerotia (Shaw, '13, pl. 8, fig. 4) discloses a type of hyphal cell not characteristic of *R. Solani;* and the small discrete sclerotia themselves (Shaw, '13, pl. 2, fig. 3, pl. 8, fig. 1) convincingly indicate that another fungus was under consideration. I can find no record of a description of sclerotia resembling these in the literature of Rhizoctonia diseases. I am at a loss to understand how a fungus with such characteristics could be likened to Kühn's fungus on the potato, even though depending upon Kühn's imperfect description. On the other hand, neither in general appearance nor in structure (as described and figured by Shaw) am I able to find any resemblance to the "small sclerotia" or infection cushions of *R. Crocorum* (*R. violacea*).

In moist situations the sclerotia of *Rhizoctonia Solani* may occur on aerial organs (as on the pods of beans, Hedgcock, '04, on lettuce leaves, Stone and Smith, '00) but the frequent and apparently normal occurrence of minute sclerotia, fairly regularly arranged, on the dead tips of stems, as described by Shaw, finds no parallel in *R. Solani.* Again, in regard to the hyphae, it may be said that while there is a characteristic location of the septum when a branch is formed in a hypha of *Rhizoctonia,* this character alone is not sufficient to identify the fungus. It is necessary to take into consideration all of the mycelial characteristics which have been referred to, and if possible also the cultural characters. The writer finds that the "Rhizoctonia type" of branching is more or less similar to that found in the hyphae of certain species of *Sclerotinia, Morchella, Pleospora, Rosellinia,* and many others. It would be unwise to offer any definite suggestions regarding the fungus described by Shaw and referred to above. What relation it may bear to the fungus of "bangle blight" (Cunning-

ham, '97) must also remain, for the present, uncertain. It is possible that Shaw's fungus is one of the *Ascomycetes,* at least this is suggested by the figures of the sclerotia.

In my opinion Shaw has correctly referred to *Corticium vagum* B. & C. (accordingly to *Rhizoctonia Solani* Kühn, representing the vegetative phases of that species) another fungus which he also found in India on the groundnut and cowpea. Both the mycelium and the sclerotia of this second organism as described by him agree with *R. Solani* as we know it on carnation, beet, bean, lettuce, potato, etc., in America and elsewhere, as far as reported. The descriptions and measurements of basidia and spores are also in sufficient accord.

Shaw has even suggested that *Rhizoctonia violacea* Tul. is the vegetative stage of *Corticium vagum* B. & C. No such unfortunate confusion could result, however, had he been able to study that which is accepted as Kühn's organism on the potato together with the violet root felt fungus of Europe on any of its hosts. He has obviously failed to find material of the last named fungus in his studies thus far.

Between *Rhizoctonia Crocorum* and *R. Solani* in the vegetative condition some of the important and easily observed contrasting features as usually found are presented in the following table:

Rhizoctonia Crocorum	Rhizoctonia Solani
An external felt, or mantle, of investing hyphae, confined almost exclusively to underground organs.	External mycelium, if noticeable, only a web, or sometimes with flaky tufts, the formation of a "collar" occurring only at the time of fruiting.
Color of mycelial felt pink-red or violet to violet-brown with age.	Color of web, if evident, dirty yellow to yellow-brown.
Protoplasm of young hyphal cells soon develops a violet reddish pigment.	Young hyphal cells hyaline, and even when flavous later, pigment confined to walls.
Infection cushions conspicuous in the root-investing mycelium on most hosts.	Nothing comparable to infection cushions, though on potato sclerotia may serve as points of infection.

Sclerotia, when present, densely wooly with investing mycelium and filaments of short, ovoidal or elliptical hyphal cells. Internal structure not truly plectenchymatic, cells variable in size.	Sclerotia normally free from any definite or permanent investment of mycelium, or filaments of elbowed hyphal cells. Internal structure homogeneous in the larger, denser sclerotia.
Cultures difficult,—not yet obtained by usual methods.	Cultures readily obtained on any nutrient medium.
Typically a parasite, with perhaps the possibility of continuing existence only for a time saprophytically.	Grows rapidly saprophytically on the invaded host, and apparently on debris in the soil when conditions are favorable.

The following species may be excluded from *Rhizoctonia* as far as can be judged from reference to the descriptions and to the exsiccati material examined:

Rhizoctonia Allii Graves, de Thuemen, Myc. Univ. Fasc. 6: *600* (obviously not closely related to the forms here discussed). *R. bicolor* Ell. N. Am. Fung. Fasc. 10: *977* (with sclerotia like those of a Botrytis, e. g., *B. cinerea*). *R. Brassicarum* Lib., Libert, Pl. Crypt. Arduennae, Fasc. 3 : *240* (no characteristics of *Rhizoctonia*). *R. muscorum* Fr. Ellis, N. Am. Fung. Fasc. 13 : *1266;* Libert, Pl. Crypt. Arduennae, Fasc. 2 : *141.*

From the descriptions alone it would seem that the following species have insufficient affinities with *Rhizoctonia* to be included, but critical study of material is needed:

Rhizoctonia aurantiaca Ell. & Ev. on decaying wood of Acer; *R. Batatas* Fr. on *Ipomoea Batatas; R. placenta* Schw., and *R. radiciformis* Schw., on decaying wood (the three last mentioned are distributed in Schweinitz', Syn. N. Am. Fung., to which, however, the writer has not yet had access); *R. destruens* Tassi, reported parasitic on five species of *Delphinium,* and on *Lobelia laxiflora,* and *Hibiscus rosa-sinensis; R. moniliformis* Ell. & Ev. on branches of *Nyssa.*

Rhizoctonia Strobi Scholz ('97) on roots of *Pinus strobus* in Austria, is insufficiently described to warrant a suggestion; and *R. subepigea* Bertoni ('97) on coffee should be included in a further comparative study.

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SOME RELATIONS OF PLANTS TO DISTILLED WATER AND CERTAIN DILUTE TOXIC SOLUTIONS

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I. Introduction

In view of the extensive use of distilled water as a medium in which to grow control plants for comparative purposes in solution-culture work, there is well-grounded justification for the performance of considerable experimental work in order to determine more definitely the relations of plants to this medium. The subject is an important one, and it will require much experimentation for the ultimate solution of all phases of the problem involved. While the results herewith reported are only preliminary in their nature, the fact that they give positive indications along certain lines has been deemed sufficient warrant for their publication at this time. In addition to determining the growth relations of plants in this and other media, consideration has also been given to the effect produced by growing plants in this medium as determined by means of electrical conductivity measurements.

II. Historical Aspects of the Subject

The relation of plants to distilled water is a matter that has been under more or less serious consideration at different periods for a long time. Woodward (1699), who first employed the method of water culture in 1691–1692 in his interesting experiments, found that plants grew better in river water than in either rain water, spring water, or distilled water. The difference was of course due to the quantity of plant food contained in the medium, and this idea, coupled also with the character of the nutrients, has been the basis for a vast amount of physiological work since that time.

Coming down to more modern times, there has been a diversity of opinion among the investigators of the subject

in regard to the reason why plants and animals thrive so much better in natural water or aqueous media than in distilled water. Considering the period from about 1860 on down to the present, the most important explanations offered may be summed up under the following three heads:

1. Lack of essential nutrients;
2. The presence of deleterious substances;
3. Extraction of salts, or nutrient materials, from the organism immersed in the distilled water.

Holding each of these views there has been a formidable array of scientists at different periods, each group contending strongly to establish the correctness of its viewpoint.

Among the earlier workers in the field may be mentioned Boehm ('75), Dehérain ('78), and others, who believed that the lack of essential nutrients in the distilled water was responsible for the resulting poor condition of the organism. Boehm, for example, believed that calcium played a fundamental rôle in the metabolism of the plant, and that in its absence certain processes, notably that of starch formation, could not be carried on and that therefore deterioration resulted. He also believed that calcium was necessary for the transfer of the reserve materials from the cotyledons to the formative organs. Dehérain repeated Boehm's experiments and confirmed his results.

Owing to the fact that even distilled water, which had been unquestioningly regarded as pure, produced effects simulating toxicity, a great deal of attention has been given in the past to the chemical and other properties of water distilled from different kinds of apparatus and under various conditions. On the animal side, workers, among whom may be mentioned Kölliker ('56) and Nasse ('69), had early noticed the injurious effects on tissues when the same were placed in distilled water. Nasse, for example, found the deleterious effect of distilled water about equal to that of the following solutions: 2.5 per cent NaCl, 3.3 per cent NaBr, 3.7 per cent Na_2SO_4, and 5.0 per cent NaI.

Nägeli ('93), in his classical work published twelve years after his death, found that very minute amounts of toxic sub-

stances, notably copper, in solution produced injurious effects on organisms (*Spirogyra*), and to this phenomenon he applied the term "oligodynamik" action. This line of work was extended to include other substances and other organisms, and claimed the attention at different times of Aschoff ('90), Loew ('91), Locke ('95), Ringer ('97), Copeland and Kahlenberg ('99), Dehérain and Demoussy ('01), Lyon ('04), Bokorny ('05), Hoyt ('13), and others. It is of particular interest to note that Ringer in some of his earlier work ascribed the injury to the extraction from the organism of necessary nutrient materials; but after the publication of Locke's experiments ('95), which Ringer duplicated and confirmed, the latter concluded that the injury done in the particular case under consideration (*Tubifex*) was due to deleterious materials in the distilled water. He says: "Copper in even infinitesimal quantities will disintegrate tubifex whilst water free from copper or other heavy metals and without any salts such as calcium salts can sustain the life of tubifex."

In regard to the third idea pertaining to the effects of distilled water on organisms, early workers, both on the plant and animal side, found that salts were extracted from organisms placed in distilled water, even though their methods for determining the extraction were somewhat crude. Among the early investigators on the animal side may be mentioned Plateau ('83), Ringer and his school ('83, '84, '85, '94, '94[a], '94[b], '97), Loeb ('03), and others. The writer has another paper ready for publication in which is given a historical treatment of the subject of excretions from roots and other plant parts, so the discussion of certain phases of the plant work is reserved for that publication.

Upon the perfection and the employment of conductivity apparatus by physical chemists, it soon began to be used also by the various workers in the fields of soil, plant, and animal investigations. In this connection distilled water came in for its share of consideration. The determination of the purity of water by ascertaining its electrical conductivity speedily came into vogue, and it should be said that as far as elec-

trolytes are concerned it is a very accurate and excellent method and has deservedly come into more and more general use for this purpose in the fields of chemistry, physics, and biology.

Koeppe ('98), for instance, determined the electrical conductivity of water obtained from various sources and compared his results with those of other workers. He believed that distilled water has a deleterious effect which is partly due to a withdrawal of salts necessary to the organism, and partly to a swelling of the tissues. He was supported in his views by Oldham ('09), while Winckler ('04), Kobert ('05), and others argued in favor of the harmlessness of distilled water, especially in medical practice. Peters ('04) used the electrolytic conductivity method in his work on *Stentor* and found that there was an exosmosis of electrolytes when the organism was placed in distilled water, and he therefore concluded that the injurious effects noted were due to an extraction of salts. True and Bartlett ('12, '15, '15[a]) considered, for certain salts, not only the excretion but also the absorption of electrolytes under balanced and unbalanced conditions of the medium.

In a recent paper in which a historical discussion of the subject is also given, True ('14) concludes that over and above any injurious effects caused by deleterious substances in the distilled water there is still a "residuum of harmful action due to no known type of impurity." Because this harmful action seems to be most marked in water of least conductivity True believes that the withdrawal of electrolytes from the root tissues best accounts for the deleterious action, but that this withdrawal is "not due to the aggregate difference in osmotic pressure between the cells of the roots and the external medium." He chose lupine seedlings for his work because Frank ('88) had found them very sensitive to distilled water. Schulze ('91), however, after several years of experience with *Lupinus luteus*, claimed that distilled water produced no toxic effects upon those plants.

Both before and after the appearance of the recent contribution by True just referred to, I carried on the investi-

gations reported in this paper, which, as previously stated, are but preliminary in their nature, but which have given indications leading to the conception of an idea differing somewhat from the majority of those above mentioned regarding the relation between plants and distilled water. This conception will be briefly mentioned here, while the evidence and a further discussion will be given later; it is that pure distilled water is not harmful or injurious *per se,* but that because of the static condition forced upon them as a consequence of the absence of plant food, the growing cells become disorganized and thus become easy prey to bacterial and fungous action. Excretion of electrolytes does occur but this should be considered merely as a concomitant condition, or resulting effect of the conditions under which the plants are placed, and should not be considered as a *cause* of degeneration unless the electrolytes themselves be toxic.

III. Methods

(Germination, Culture, and Conductivity)

Canada field peas (*Pisum sativum*) and horse beans (*Vicia faba*), the small variety, were the plants selected, as both were known to be well adapted for growth in solution cultures. Of the various methods of seed sterilization tried out, the one in which the seeds were treated with 1–600 formalin-water for 15 minutes after being soaked for 24 hours in running water gave best satisfaction.

For germinating the seeds a modification of the method used by Boussingault ('74), and also by various investigators in the Bureau of Soils, was employed. This consisted in the use of ordinary enameled-ware pans about 12 inches in diameter and 3 inches in height, filled with tap water and covered with 6 × 6-mesh galvanized iron "hardware cloth," on which the previously soaked and sterilized seeds were placed. The seeds were then covered with filter paper or paper towelling which was kept moist throughout the germination process or until the radicles reached the water below. The germination was carried on in the greenhouse. In the

course of four or five days a splendid lot of vigorous, uniform seedlings which have serviceably straight radicles about 2 inches long with no laterals yet formed is obtained by this method; such seedlings are well adapted, both by their character and their accommodation to an aqueous medium, for solution-culture work. At this stage the plumules have grown to about one-half inch in length, and the plants are now ready for transfer to the culture medium, an operation which is easily and quickly done. This method of germination, which is shown in pl. 16 fig. 2, recommends itself both by reason of its simplicity and ease of operation and the certainty of securing excellent results. In the transfer process from the germinating pan to the culture medium, the entire seedling was always immersed and carefully rinsed in once-distilled and again in twice-distilled water; by this means the roots became free of any adhering impurities.

As containers for the cultures, ordinary glass tumblers were used, the sides of which were covered with black paper to prevent algal growth and the top covered with perforated paraffin paper. (For a complete description and illustration of the method see the paper by McCool, '13.) Ten plants were grown in most cases in each tumbler; exceptions to that number will be noted in each case when the series are discussed in detail. Galvanized iron wire supports were used to hold the plants upright when the seedlings had attained sufficient size to require them.

In all cases doubly distilled water was used, the second distillation being carried out in the laboratory with $KMnO_4$ added to the once-distilled water to oxidize any organic matter that might be present. Conductivity tests of this water showed it to possess a specific conductivity of 2.064×10^{-6}. The nutrient solution used was that of Pfeffer, redistilled water being the solvent for the necessary salts. Each tumbler was filled to a convenient level with either the water or the full nutrient solution as the case might be, approximately 250 cc. being required. To replace transpiration loss, doubly distilled water was added as needed.

In the early days of conductivity work on solutions,

measurements could be made only by means of a continuous current. Because of the resulting polarization effects, however, the resistance of the solution increased to such an extent as to introduce serious errors into the results. But thanks to the classical work of Kohlrausch and others, the alternating current method was devised and perfected, whereby the determinations became practically independent of polarization effects. A vast amount of work has since been done in the realm of physical chemistry on conductivity measurements, a review of which, however, is outside the scope of this paper. For a clear and concise discussion of this subject see Jones ('09), Walker ('10), or Findlay ('10).

In addition to the investigations already cited which deal with the practical applications of conductivity work, there might well be mentioned in this connection the work done by investigators in the Bureau of Soils of the U. S. Department of Agriculture: Whitney, Gardner, and Briggs ('97); Whitney and Briggs ('97); Whitney and Means ('97); and Gardner ('98). Heald ('02) used the Kohlrausch method for determining the conductivity of plant juices in order to get indications regarding the dissolved mineral substances in different parts of the plants under experimentation. Nicolosi-Roncati ('07), Bouyoucos ('12), Dixon and Atkins ('13, '13[a]) and others have also carried on conductivity determinations with different plants and under various conditions.

Sjöqvist ('95) was the first to use the conductivity method in enzyme investigations, which he did in his work on the action of pepsin on protein solutions. Similar work was done by Oker-Blom ('02), who also extended the applications of this method. Oker-Blom ('12) has recently given an account of his own and previous investigations in the field of bacteriology, wherein the electrical conductivity method was used. Various other investigators have also made use of it, among whom may be mentioned Bayliss ('07). Stiles and Jörgensen ('14) give a partial review of some of the historical aspects of this subject as it pertains to plant work.

For the conductivity work herein reported the following apparatus was used:

Wheatstone bridge (Central Scientific Co., catalogue number, 2475);
Resistance box, 11,110 ohms (Central Scientific Co., catalogue number, 2444);
Induction coil (Einer and Amend, catalogue number, 4100);
Dry battery cells (Einer and Amend, catalogue number, 592);
Conductivity cell, Freas (Einer and Amend, catalogue number, 5202);
Telephone receiver (Central Scientific Co., catalogue number, 2355);
Thermometer graduated to 1/10°C;
Water tank holding 50 gallons, specially constructed for the purpose, pilot flame underneath; temperature regulated to 1/10°C;
Tiffany laboratory motor with which to operate a stirring apparatus in the tank.

In the method employed for the work the procedure given below was consistently followed: the tumblers were always filled to approximately the 250 cc. level with either the solution or redistilled water, depending on the culture. Before taking readings, doubly distilled water was added to bring the water or solution up to the original level, if the transpiration loss since the previous reading made the addition necessary. This was of course essential in order to keep the concentration factor under control. Readings in all cases were taken at 25 °C. The control of temperature exactly to within 1/10°C. was comparatively easy by the use of the pilot flame underneath and the stirring apparatus in the tank of water.

For absolutely accurate and final quantitative determinations or ultimate values, as were required, for example, in the case of the standardization measurements for the cell constant with N/50 KCl, or the determination of the specific conductivity of the doubly distilled water, the greatest precautions possible were taken in regard to the conductivity cell and the concentration of its contents. But in making hundreds and even thousands of determinations, most of them as rapidly as accuracy permitted, due to the time factor involved, it was both impossible and unnecessary to dry the cell after each reading, since relative, and not absolute, values were

desired for the most part. The method employed, therefore, was to remove from the carefully stirred solution in the tumbler a 25 cc. sample with a pipette of the same capacity, the latter having previously been rinsed with the solution. Using exactly the same amount for each determination further reduced any possibility of error due to unequal dilution in the conductivity cell. Between readings the pipette was kept almost entirely immersed in redistilled water in a tall cylinder attached to a stand in the water bath. After carefully pouring the sample back into the tumbler, in case further readings were to be taken, the cell was rinsed twice with doubly distilled water and rapidly drained before taking the next reading, whether of the same or of a different culture. Any minute amount of doubly distilled water that might be present to dilute the next sample was a constant factor throughout all the readings and was of course inconsequential.

In using fresh batteries it was necessary to insert resistance coils between the battery and the induction coil in order to reduce the current. For this purpose German silver wire was used. While polarization phenomena may possibly be operative to a certain extent, such would be so small as to be practically negligible, especially in view of the fact that the effects from such a cause would be entirely relative and would therefore not affect the validity of the results.

Some of the conductivity results given in this paper are shown in tabular form and others are plotted as curves. In some instances the data are calculated as specific conductivity; in other cases the conductivity is represented by the value of x on the Wheatstone bridge. To make it clear what x actually represents, when the apparatus is set up as it was for the determinations, the following proportion is given:

$$R : R' :: x : 100—x$$

R is the resistance in ohms inserted in the resistance box; R′ is the resistance in ohms of the solution; and x is the number on the bridge wire (graduated in millimeters from 0 to 100 centimeters). As the position of x on the bridge varies with R and R′, the R for each series of curves or tables will be given (though in the great majority of cases it was 9,110),

from which R′ can then be calculated. Having these values, the specific conductivity can be calculated for any determined cell constant (the value of the cell used being .4088). For a fuller discussion see Findlay ('10).

IV. Recovery of Plants after being in Distilled Water for Varying Periods

The first question studied pertained to the recovery of plants in full nutrient solution after being kept in doubly distilled water for varying periods. To determine the comparative condition for optimum recovery, the distilled water and the full nutrient medium were renewed every four days in some cultures and left unrenewed in others, in such a way that for either condition of the medium of each set in a certain period the other medium would be both renewed and unrenewed so as to give all possible methods of combination. Examination of table 1 will make this clear. Thus, for example, with cultures 11, 12, 13, and 14 of the 10-day period in distilled water the doubly distilled water in Nos. 11 and 12 was unrenewed; but when these cultures were placed in full nutrient solution this medium was unchanged or unrenewed for No. 11 and was renewed for No. 12. The distilled water in Nos. 13 and 14 was renewed, and the full nutrient solution unrenewed and renewed respectively.

In series 1 the small variety of horse beans (*Vicia faba*) was employed, 8 plants being used to a culture. The condition of the media and duration of growth, the green weight of tops, and the dry weight of tops and roots of series 1 are given in table 1. On examining this table it is seen that even after the plants had remained for 20 days in distilled water, they recovered on being placed in the full nutrient solution, while those remaining for 10 days in distilled water produced practically as much growth when later placed in the full nutrient solution as did the plants which were in the latter medium during the entire period.

Of course, as would be expected, the cultures wherein the full nutrient solution was renewed every four days gave much better growth than did those in the unrenewed medium, due,

no doubt, to an increased amount of available nutrients. But an interesting comparison is manifest in connection with the effect of renewing the distilled water; the greater growth of both tops and roots may be noted in cultures 3 and 4, in which

TABLE I (Series 1)

EFFECT OF RENEWED VS. UNRENEWED MEDIA ON GROWTH OF HORSE BEANS

Culture no.	Length of period in dist. H_2O days	Dist. H_2O renewed or unrenewed	Length of period in full nutr. days	Full nutr. renewed or unrenewed	Green wt. of tops gms.	Dry wt. of tops gms.	Dry wt. of roots gms.
1	45	Unrenewed	.....	.....	2.15	.777	.124
2	45	Unrenewed	.....	.....	1.75	.666	.096
3	45	Renewed	.....	.....	4.40	.887	.272
4	45	Renewed	.....	.....	4.40	.870	.235
5	1	Unrenewed	44	Unrenewed	16.30	2.069	.485
6	1	Unrenewed	44	Renewed	27.00	3.069	.707
7	2	Unrenewed	43	Unrenewed	15.85	1.994	.429
8	2	Unrenewed	43	Renewed	32.51	3.543	.743
9	5	Unrenewed	40	Unrenewed	18.90	2.315	.463
10	5	Unrenewed	40	Renewed	26.25	2.895	.700
11	10	Unrenewed	35	Unrenewed	13.35	1.623	.382
12	10	Unrenewed	35	Renewed	26.35	2.928	.697
13	10	Renewed	35	Unrenewed	14.90	1.887	.463
14	10	Renewed	35	Renewed	22.90	2.376	.548
15	15	Unrenewed	30	Unrenewed	14.00	1.719	.388
16	15	Unrenewed	30	Renewed	18.05	1.880	.457
17	15	Renewed	30	Unrenewed	15.60	1.807	.417
18	15	Renewed	30	Renewed	21.20	2.403	.642
19	20	Unrenewed	25	Unrenewed	12.51	1.447	.300
20	20	Unrenewed	25	Renewed	11.40	1.247	.328
21	20	Renewed	25	Unrenewed	11.40	1.319	.284
22	20	Renewed	25	Renewed	15.50	1.670	.454
23	.....	.....	45	Unrenewed	14.60	1.975	.419
24	.....	.....	45	Unrenewed	14.70	2.044	.443
25	.....	.....	45	Renewed	27.85	2.925	.690
26	.....	.....	45	Renewed	27.05	3.025	.764

the distilled water was renewed every four days throughout the period, as compared with that of cultures 1 and 2, where the distilled water was not renewed, except, of course, for the occasional addition of water to replace the transpiration loss, which, however, was small. Furthermore, in noting the growth of cultures 11–22 inclusive, it is seen that in four of the six cases of comparison between the renewed and unrenewed distilled water, better growth of both tops and roots resulted where the distilled water was renewed. Considering cultures 1–4 and 11–22, inclusive, the total green weight of tops for the unrenewed distilled water as compared with the

renewed distilled water, and the same conditions for the dry weight of tops and roots, gave the results to be seen in table II. The total weight in all cases is therefore greater in the cultures in which the distilled water was renewed.

TABLE II (Series 1)
EFFECT OF RENEWED VS. UNRENEWED DISTILLED WATER ON GROWTH OF HORSE BEANS
(Summarized Results of Part of Table I)

Medium	Green wt. of tops in grams	Dry wt. of tops in grams	Dry wt. of roots in grams
Water renewed...	110.30	13.219	3.315
Water unrenewed	99.56	12.287	2.772

These results therefore indicate that the so-called injury to plants in distilled water cannot be entirely or even satisfactorily explained on the basis of extraction of solutes from the plant tissues. If that were the case we should have the greatest injury and least recovery in those cultures in which the distilled water was renewed, the periodically renewed water effecting *in toto* a greater exosmosis of the salts than the water which is not renewed. This statement will receive verification under the section on conductivity measurements. It would therefore seem that we must seek other explanations for the phenomena observed when plants are placed in distilled water. This phase of the subject will also be discussed later.

The points noted will be clear from an examination of pl. 13 figs. 1 and 2. Plate 13 fig. 1 shows the various stages of recovery after varying periods in the distilled water. The better growth is to be noted of both tops and roots of No. 2, in which the distilled water was renewed, as contrasted with No. 1, in which it was not renewed. It is interesting to observe how plants, even after 20 days in distilled water, will recover in full nutrient solution and then give even better growth than plants in unrenewed full nutrient solution the entire period, and that after 10 days in distilled water, plants will recover in renewed full nutrient solution and equal in

growth, plants grown the entire period in renewed full nutrient solution.

Plate 13 fig. 2 shows first (Nos. 1 and 2) the contrasted effect of renewing and not renewing the full nutrient solution. The remaining 8 cultures of the plate show the effect of renewing and of not renewing both the distilled water and the full nutrient solution. In cultures 3–10 the comparison should, of course, be made between the alternating numbers for the distilled water effect (renewed or not renewed), and between successive numbers for the effect of the renewal or the non-renewal of the full nutrient solution. While the culture represented by No. 7 of the plate gave greater growth than did No. 9, that excess was probably due to the individual hardihood of two plants. It is seen that a much more uniform and desirable growth was made by the plants of No. 9.

An interesting point in connection with the horse beans is that 16 days after setting up the series the tips of those plants still in distilled water were more or less blackened, probably as a result of enzyme (oxydase) action, and many of them were considerably inrolled. Such conditions were entirely absent from the cultures in full nutrient solution at that time. When the affected plants were later placed in full nutrient solution there was a gradual recovery from the blackening of the leaves, and this recovery was greater in the case of those cultures in which the distilled water had been renewed than in those in which it had not been renewed. Twenty days later Nos. 3 and 4 were in very much better condition than Nos. 1 and 2. There was much less blackening, some leaves not being blackened at all. The general height of the plants in Nos. 1 and 2 was $1\frac{1}{2}$–$2\frac{1}{2}$ inches; and in Nos. 3 and 4 it was $2\frac{1}{2}$–4 inches. A very noticeable feature at the end of the experiment was the condition of the medium, that of Nos. 3 and 4 being of course clear while that of Nos. 1 and 2 was milky, turbid, and opaque, indicating abundant fungous and bacterial action, a condition further emphasized by the hyphal threads and gelatinous coating on the roots.

The roots of the plants in Nos. 3 and 4 were also in much better condition at the end of the experiment than were those

of Nos. 1 and 2, especially as regards length and the amount of lateral root development. The root growth in No. 13 at the end of the experiment was also greater than that in No. 11; but in Nos. 12 and 14 it was about equal. The plants of No. 17 also showed greater root growth than did those of No. 15, and this difference was more marked than in the case of the tops. The lateral roots in No. 17 were produced all along the main roots, while in No. 15 they were practically confined to the upper or older portion of the main roots. Another interesting difference observed was that in No. 17 the main root tips were not permanently injured in the distilled water and when placed in the full nutrient solution they continued growth. This was not the case in No. 15. In general there was not much difference between the roots in Nos. 16 and 18; the plants in No. 18, however, had slightly greater growth of roots and showed less injury and some continuation of growth of the tips, whereas those in No. 16 did not. The same condition of the roots above noted for Nos. 15 and 17 held also in Nos. 19 and 21 respectively; but the difference in favor of the renewal of the distilled water though less marked was nevertheless evident. Likewise, Nos. 18 and 20 were similar to Nos. 16 and 18 respectively.

Strong evidence was therefore afforded by the cultures of horse beans that renewing the distilled water has a favorable effect upon the plants.

Series 2 is in every respect a duplicate of series 1 except that Canada field peas (*Pisum sativum*) were used instead of horse beans (*Vicia faba*), and that the dry weight of the tops was not determined; furthermore, the length of the experimental period was different. The condition of the media and duration of growth, the green weight of tops, and the dry weight of roots are given in table III, seven plants being grown in each culture. An examination of this table reveals results similar in many cases to those contained in table I; plants recovered even after 20 days in distilled water, but after 10 days in this medium the recovery was not so complete as in the case of the horse beans, for the plants so treated did not equal in growth similar ones which had remained in

full nutrient solution the entire period. However, plants which had been in distilled water only 5 days before being transferred to full nutrient solution subsequently equalled in growth other plants which had been in the latter medium from

TABLE III (Series 2)

EFFECT OF RENEWED VS. UNRENEWED MEDIA ON GROWTH OF PEAS

Culture no.	Length of period in dist. H_2O days	Dist. H_2O renewed or unrenewed	Length of period in full nutr. days	Full nutr. renewed or unrenewed	Green wt. of tops gms.	Dry wt. of roots gms.
1	47	Unrenewed	.....	.....	.80	.073
2	47	Unrenewed	.....	.....	.60	.076
3	47	Renewed	.....	.....	.45	.067
4	47	Renewed	.....	.....	1.30	.091
5	1	Unrenewed	32	Unrenewed	6.75	.400
6	1	Unrenewed	32	Renewed	11.05	.500
7	2	Unrenewed	31	Unrenewed	5.50	.401
8	2	Unrenewed	31	Renewed	12.90	.568
9	5	Unrenewed	28	Unrenewed	5.95	.263
10	5	Unrenewed	28	Renewed	12.35	.467
11	10	Unrenewed	23	Unrenewed	5.35	.254
12	10	Unrenewed	23	Renewed	7.90	.321
13	10	Renewed	23	Unrenewed	3.80	.160
14	10	Renewed	23	Renewed	6.07	.202
15	15	Unrenewed	18	Unrenewed	3.30	.144
16	15	Unrenewed	18	Renewed	4.40	.175
17	15	Renewed	18	Unrenewed	4.30	.162
18	15	Renewed	18	Renewed	4.15	.169
19	20	Unrenewed	13	Unrenewed	3.21	.124
20	20	Unrenewed	13	Renewed	2.52	.092
21	20	Renewed	13	Unrenewed	4.05	.139
22	20	Renewed	13	Renewed	4.43	.141
23	.....	.....	33	Unrenewed	5.50	.368
24	.....	.....	33	Unrenewed	6.94	.420
25	.....	.....	33	Renewed	9.60	.536
26	.....	.....	33	Renewed	13.45	.611

the start. The period between 5 and 10 days in distilled water is therefore a critical one, and will be discussed later in other connections.

Renewing the full nutrient solution again showed beneficial results, as might be expected. But the renewal of the distilled water did not produce such striking results in some respects as in the case of the horse beans; in other ways, however, the results were equally or even more striking. Where the plants remained in distilled water for 47 days the growth was better in one case and poorer in the other where the distilled water was renewed than where it was not renewed. The average

growth, however, of the two cultures in the renewed medium was better than that of the two in the unrenewed distilled water.

In Nos. 11–22, there was better growth of tops and roots

TABLE IV (Series 2)

EFFECT OF RENEWED VS. UNRENEWED DISTILLED WATER ON GROWTH OF PEAS

(Summarized Results of Part of Table III)

Medium	Green wt. of tops in grams	Dry wt. of roots in grams
Distilled water renewed...	28.55	1.131
Distilled water unrenewed.	28.08	1.259

in four cases where the distilled water was renewed and better growth in four cases where it was not renewed. Considering cultures 1–4 and 11–22 the results given in table IV were obtained, from which it is again evident that renewing the distilled water exercises no injurious influence, and the conclusion is reinforced that an exosmosis of mineral nutrients is not the fundamental basis of the injury which plants suffer in distilled water. Furthermore, the difference between the renewed and the unrenewed distilled water cultures was very marked if the plants remained for 20 days in distilled water before being changed to the full nutrient solution, the difference being greatly in favor of the cultures in which the medium was renewed.

Figures 1 and 2 of pl. 14 illustrate the points above mentioned. In pl. 14 fig. 2 should be noted the better growth of Nos. 9 and 10—which were in renewed distilled water for 20 days before transfer to full nutrient solution—as compared with Nos. 7 and 8, which had remained in unrenewed distilled water for the same length of time before transfer. The excess of growth in No. 4 over that in No. 6 is probably to be accounted for on the ground that since those cultures were in distilled water but 10 days neither the renewal nor the unrenewal of the medium exercised much effect. Hence the greater growth of No. 4 represents an individual variation.

At the expiration of the experimental period the following conditions prevailed in series 2: while the top growth in

cultures 1–4 was about the same in each case, the root growth in Nos. 3 and 4 was much better than that in Nos. 1 and 2, the roots of the former being whiter, cleaner, and having longer and more numerous lateral roots. In the case of those cultures grown in distilled water 10 days before removal to full nutrient solution, Nos. 11 and 12 were in somewhat better condition than Nos. 13 and 14, a difference which might readily be expected for the shorter periods in distilled water due to individual variation. After 15 days in distilled water and 18 days in full nutrient solution the benefits derived from renewing the former were markedly evident in the appearance of cultures 15–18, even though the actual weights did not show such difference. Nos. 17 and 18 were in better condition than Nos. 15 and 16 respectively, especially as regards the root growth; similarly, Nos. 21 and 22 were in better condition than Nos. 19 and 20 respectively.

Some special conditions which are of particular interest were observed when the cultures were examined carefully at the close of the experiment. The first point pertains to the method of recovery. After being in the distilled water only one or two days the top growth of such cultures when placed in full nutrient solution proceeds unhindered from the tips of the main stems, i. e., the tips of the stems remain uninjured and resume growth. But 5 days in distilled water almost marks the limit at which growth can be resumed at the tip of the main axis of the stem when such cultures are subsequently placed in full nutrient solution. After 10 days in distilled water the tips of the stems become injured so that the later growth in full nutrient solution is made from new lateral branches. Hence the period from 5 to 10 days in distilled water before removal to full nutrient solution may be considered a crucial period as regards the recovery and growth of the main stems.

Another point of interest is the delayed maturity which results in the case of the cultures which are grown for some time in distilled water and later are placed in full nutrient solution. Such plants remain in a green and growing condition much longer than do those which have been in full

nutrient solution for the entire period, or those which remained in distilled water for a shorter period before being transferred to the full nutrient solution. The growing season of the former is thus prolonged and the date of maturity delayed.

The foregoing series having given evidence of the recovery of plants in full nutrient solution after being in distilled water for 20 days, the question arose as to the maximum length of time plants might remain in distilled water without preventing recovery when subsequently transferred to full nutrient solution. Series 3 was therefore set up. This consisted of cultures of Canada field peas grown in distilled water for 10, 20, 30, 40, and 50-day periods before transfer to the full nutrient medium. The condition of the media and duration in each and also the results of the series (as shown by the green weight of tops) are given in table v, Nos. 1–20 inclusive. Renewals in this series also were made every four days. Nos. 21–28 under different conditions and concentration of nutrient solution are given for purposes of comparison. The maximum time limit in distilled water above referred to is thus seen to be approximately 30 days, and this was practically attained only in case of the cultures in renewed distilled water. After 40 days in distilled water, whether renewed or unrenewed, the recovery was almost nil, though somewhat better in the renewed, while after 50 days in either renewed or unrenewed distilled water all the cultures were dead.

In the 10 cases furnishing comparisons between cultures in which the full nutrient solution was preceded on the one hand by renewed and on the other by unrenewed distilled water, greater growth was attained in 7 cases where the distilled water was renewed. The total weight of green tops is more nearly equal in the two sets of cultures, however, being 24.20 grams in the case of those in the unrenewed and 22.38 grams in the case of those in the renewed distilled water. We thus see that no injurious effects attend the renewal of the distilled water when compared with the non-renewal of the same; on the other hand, positive benefits are derived from such a

renewal, especially in the case of plants approaching the maximum time limit of durability in distilled water—a period which enables the results of the two conditions to be more readily seen and compared.

TABLE V (Series 3)

GROWTH OF PEAS IN RENEWED AND UNRENEWED MEDIA FOR VARIOUS PERIODS UP TO THE MAXIMUM TIME FOR SURVIVAL. ALSO EFFECT OF ADDING WATER AT DIFFERENT INTERVALS TO MEDIA UNDER VARIOUS CONDITIONS

Culture no.	Length of period in dist. H_2O days	Dist. H_2O renewed or unrenewed	Length of period in full nutr. days	Full nutr. renewed or unrenewed	Green wt. of tops gms.
1	10	Unrenewed	42	Unrenewed	4.50
2	10	Unrenewed	42	Renewed	8.00
3	10	Renewed	42	Unrenewed	4.85
4	10	Renewed	42	Renewed	6.30
5	20	Unrenewed	32	Unrenewed	2.70
6	20	Unrenewed	32	Renewed	5.05
7	20	Renewed	32	Unrenewed	3.55
8	20	Renewed	32	Renewed	1.40
9	30	Unrenewed	22	Unrenewed	.55
10	30	Unrenewed	22	Renewed	1.30
11	30	Renewed	22	Unrenewed	1.90
12	30	Renewed	22	Renewed	1.90
13	40	Unrenewed	12	Unrenewed	.50
14	40	Unrenewed	12	Renewed	.55
15	40	Renewed	12	Unrenewed	.65
16	40	Renewed	12	Renewed	.72
17	52	Unrenewed	.....	.....	.60
18	52	Unrenewed	.....	.....	.45
19	52	Renewed	.....	.....	.56
20	52	Renewed	.....	.....	.55
21	Unrenewed full nutr. 42 days, dist. H_2O added every 8 days				6.40
22	Unrenewed full nutr. 42 days, dist. H_2O added every 4 days				6.00
23	Renewed full nutr. 42 days, the sol'n. renewed every 8 days				8.75
24	Renewed full nutr. 42 days, the sol'n. renewed every 4 days				18.50
25	Unrenewed 1/10 full nutr. 42 days, dist. H_2O added every 4 d'ys				2.90
26	Unrenewed 1/5 full nutr. 42 days, dist. H_2O added every 8 days				2.95
27	Renewed 1/10 full nutr. 42 days, sol'n. renewed every 4 days				10.10
28	Renewed 1/5 full nutr. 42 days, sol'n. renewed every 8 days				7.85

In pl. 15 fig. 1 some of the cultures are illustrated, the ones of special interest being Nos. 9–14. The exceptionally small or irregular growth of No. 8 is difficult to account for, because in the renewed full nutrient it should be greater than that of No. 7. Individual resistance is apparent, however.

V. Recovery of Plants after being in Toxic Solutions

Having thus ascertained the maximum time plants may remain in distilled water and then recover on being placed in full nutrient solution, we may turn our attention to toxic solutions. If distilled water in itself is toxic then it should be interesting to get quantitative data on its effects as measured by the power of plants so treated to recover. This power should furnish a good index regarding the extent of any injury suffered. By comparing the ultimate time limits for various media after which recovery in full nutrient solution is possible, we are able to get a basis on which to determine the relative toxicity of each medium. Almost simultaneously with series 3, series 4 was set up. The plan of the series and the green weight of tops and dry weight of roots of the plants in series 4 are given in table VI, while pl. 15 fig. 2 shows the actual condition of the plants in some of the media. The results obtained indicate the following relative toxicities of the substances used, the time expressed in days having reference to the longest period in the toxic solution after which recovery is possible:

Redistilled water	30–40 days
N/100 $MgCl_2$	4–8 days
N/1000 $MgCl_2$	about 20 days
N/1000 $CaCl_2$ & N/20 $MgCl_2$	about 16 days
N/12800 H_2SO_4	about 20 days
N/400 KOH	about 20 days

We thus see that as compared with the toxic solutions mentioned distilled water, if it be considered as a toxic agent at all, is much less so than either of the others given above. In this connection it is interesting to note that Kahlenberg and True ('96) found that N 12800 H_2SO_4 and N 400 KOH were approximately the critical concentrations for Lupinus roots. Hence, the fact that plants can remain much longer in distilled water than in these solutions and still recover would seem to indicate that as regards toxicity distilled water is only very slightly if at all deleterious. But the writer believes that it is entirely incorrect and misleading to speak of distilled water as being toxic. What is illustrated above for distilled water is not toxicity, therefore, but merely the length of time

TABLE VI (Series 4)

EFFECT ON GROWTH OF PLANTS OF VARIOUS PERIODS IN TOXIC SOLUTIONS

Culture no.	First sol'n. or medium	Length of period in first medium days	First medium renewed or unrenewed	Length of period in full nutr. days	Green wt. of tops gms.	Dry wt. of roots gms.
1	Dist. H_2O	32	Unrenewed		1.15	.116
2	Dist. H_2O	32	Renewed		1.55	.130
3	N/100 $MgCl_2$	32	Unrenewed		.35	.012
4	N/100 $MgCl_2$	32	Renewed		.40	.016
5	N/100 $MgCl_2$	1	Unrenewed	31	10.15	.428
6	N/100 $MgCl_2$	2	Unrenewed	30	8.40	.372
7	N/100 $MgCl_2$	4	Unrenewed	28	5.15	.132
8	N/100 $MgCl_2$	8	Unrenewed	24	.35	.018
9	N/100 $MgCl_2$	12	Unrenewed	20	.30	.020
10	N/100 $MgCl_2$	16	Unrenewed	16	.40	.016
11	N/100 $MgCl_2$	20	Unrenewed	12	.28	.012
12	N/1000 $MgCl_2$	32	Unrenewed		1.00	.085
13	N/1000 $MgCl_2$	32	Renewed		1.00	.038
14	N/1000 $MgCl_2$	2	Unrenewed	30	8.85	.385
15	N/1000 $MgCl_2$	4	Unrenewed	28	9.70	.384
16	N/1000 $MgCl_2$	8	Unrenewed	24	7.20	.305
17	N/1000 $MgCl_2$	12	Unrenewed	20	5.15	.192
18	N/1000 $MgCl_2$	16	Unrenewed	16	2.05	.121
19	N/1000 $MgCl_2$	20	Unrenewed	12	1.05	.093
20	N/1000 $CaCl_2$ and N/20 $MgCl_2$	32	Unrenewed		.75	.092
21	N/1000 $CaCl_2$ and N/20 $MgCl_2$	32	Renewed		.85	.099
22	N/1000 $CaCl_2$ and N/20 $MgCl_2$	1	Unrenewed	31	10.60	.409
23	N/1000 $CaCl_2$ and N/20 $MgCl_2$	2	Unrenewed	30	9.35	.388
24	N/1000 $CaCl_2$ and N/20 $MgCl_2$	4	Unrenewed	28	10 35	.384
25	N/1000 $CaCl_2$ and N/20 $MgCl_2$	8	Unrenewed	24	8.40	.294
26	N/1000 $CaCl_2$ and N/20 $MgCl_2$	12	Unrenewed	20	3.00	.144
27	N/1000 $CaCl_2$ and N/20 $MgCl_2$	16	Unrenewed	16	1.50	.117
28	N/1000 $CaCl_2$ and N/20 $MgCl_2$	20	Unrenewed	12	.75	.103
29	Full nutr. sol'n.	32	Unrenewed	32	8.95	.411
30	Full nutr. sol'n.	32	Renewed	32	18.50	.530
31	N/12800 H_2SO_4	32	Unrenewed		1.55	.130
32	N/12800 H_2SO_4	32	Renewed		1.25	.124
33	N/12800 H_2SO_4	2	Unrenewed	30	7.05	.318
34	N/12800 H_2SO_4	8	Unrenewed	24	7.45	.289
35	N/12800 H_2SO_4	16	Unrenewed	16	4.40	.236
36	N/12800 H_2SO_4	20	Unrenewed	12	1.95	.172
37	N/400 KOH	32	Unrenewed		1.25	.094
38	N/400 KOH	32	Renewed		1.50	.108
39	N/400 KOH	2	Unrenewed	30	8.60	.444
40	N/400 KOH	8	Unrenewed	24	6.60	.214
41	N/400 KOH	16	Unrenewed	16	2.55	.092
42	N/400 KOH	20	Unrenewed	12	2.60	.117

plants can survive in a medium without nutrient materials. That these plants could not survive for that length of time in the other media, however, shows that in those cases a real toxicity enters into consideration.

In addition to the actual time limits for recovery just tabulated, as well as the method of recovery and delayed maturity mentioned in the preceding section, another interesting point, which was very noticeable in the cultures and which can also be seen in the plates, is the character of growth of the rootlets in the boundary cultures, by which is meant those cultures which have remained in the inimical media nearly as long as their endurance would permit, and whose recovery in full nutrient solution is slower or more difficult than the normal unaffected plants. In the latter case the roots are short and compact and usually extend down only to about one-half the distance to the bottom of the tumbler. In the case of the first mentioned cultures, however, when transferred to full nutrient solution the rootlets develop a long, slender growth easily extending to the bottom of the tumbler.

VI. Effect of Sterilizing the Water During Growth of Plants

The foregoing series pointed, therefore, to factors other than extraction or loss of solute from the plant tissue as being responsible for the deteriorating phenomenon observed when growing plants are placed in distilled water. In the unrenewed water cultures in the previous series a brownish coloration developed and the roots appeared, in their gelatinized condition, to be covered by bacterial and fungous growths. Suspecting that these organisms played an important rôle, it was decided to grow additional cultures to test this point. Four cultures, each containing ten plants of *Pisum sativum*, were set up in distilled water: in one the medium was not renewed; in a second the water was renewed every four days; and in the remaining two the medium was sterilized every four days by boiling in a return condenser one-half hour. The results are given in table VII (series 5) and the cultures are shown in pl. 16 fig. 1. The full nutrient solution cultures

were grown for purposes of comparison. The duration of growth was 30 days.

Whether the beneficial effect of the sterilization was due to the destruction of the bacterial and fungous floras of the

TABLE VII (Series 5)

EFFECT PRODUCED ON GROWTH OF PLANTS BY STERILIZING THE WATER IN WHICH THEY ARE GROWN

Culture no.	Medium	Condition of medium	Green wt. of tops gms.	Dry wt. of roots gms.
1	Dist. H_2O	Unrenewed	1.55	.141
2	Dist. H_2O	Renewed	1.65	.150
3	Dist. H_2O	Sterilized	2.40	.225
4	Dist. H_2O	Sterilized	3.05	.233
5	Full nutr.	Unrenewed	10.30	.342
6	Full nutr.	Renewed	17.65	.507

medium, to a decomposition of any contained toxic substances (thereby rendering them less toxic), or to incidental effects such as aëration of the water by the boiling process, was not definitely determined. Neither was this effect compared with that produced by the addition of various bodies (tannic acid, pyrogallol, calcium carbonate, various hydrates, carbon black, and other substances mentioned by Livingston and his co-workers, '05, '07, Dachnowski, '08, '09, and others). In the last paper of Livingston and his co-workers referred to are given the results of boiling the aqueous extracts from soils containing toxic properties as determined by the growth of plants in the same. The boiling improved the extracts, but this effect was explained by "supposing the process of boiling to remove or change the toxic action of this extract, the toxic materials being perhaps partly volatile with steam." But since in our sterilization process a return condenser was used the removal of toxic substances by volatilization would not occur. A breaking down of toxic compounds into less toxic constituents may possibly be a condition induced by the boiling, however. It will be recalled that Lyon ('04) found the toxicity of tap water reduced by boiling.

While the oxidizing power of roots, due to enzymatic activity, may be an important factor in aiding in the decom-

position of vegetable matter in the soil, as pointed out by Schreiner and Reed ('07) and others, it is not believed that in the case under consideration the oxidizing power of the roots was altered to any appreciable degree by the boiling of the medium. Dachnowski ('12) mentions the effect of oxidation upon the toxic substances found in bog water. In the sterilization method by boiling under a return condenser, however, the aëration or oxidation phenomenon would no doubt play only a subsidiary rôle. The stronger line of evidence seems to favor the destruction of injurious bacterial and fungous agencies as the chief factor in the beneficial effect of the sterilization.

VII. Conductivity Measurements

The excellence of the electrical conductivity method for determining any change in the electrolyte content of an aqueous medium naturally led to its adoption for the experimental work described below. This phase of the investigation was especially concerned with determinations pertaining to the extraction of electrolytes — including the essential nutrient salts—from the roots of plants in distilled water. The generally beneficial results attendant upon a frequent renewal of the distilled water in which the plants were placed has already been noted, as well as the evidence in favor of the view that conditions other than extraction of essential salts constitute the underlying cause of the deterioration of plants in distilled water.

The next point to be determined was the relative amount of the total exosmosis in the renewed distilled water as compared with that in the unrenewed. In placing roots in distilled water it is pertinent to this subject to inquire whether all the exosmosis occurs during the first four days. If it does, we should have the same amount of extraction in both the unrenewed water and that renewed every four days. Or is there a renewal of the exosmosis of the electrolytes following the renewal of the water each time, thereby giving rise to a greater exosmosis than in the cultures in which the water was not renewed? If such a condition obtains and yet in

spite of it the renewal of the water shows no baneful effects, or indeed produces beneficial results, then may we well conclude, and with increasing assurance, that extraction of nutrient salts is in no way responsible for any injury plants undergo in distilled water. The results obtained strongly substantiate that conclusion.

A series of cultures (series 6) was set up in which healthy plants of Canada field peas were grown in full nutrient solution for about three weeks and then transferred, after carefully rinsing the roots, to doubly distilled water. In half of the cultures the distilled water was renewed at certain definite intervals for each culture, while in the other half of the cultures the water was not renewed. Conductivity determinations were then made of the water under both conditions—renewal and non-renewal—at certain regular intervals, varying for each set of cultures, for several days after the plants had been placed in this medium.

By numerous readings it was ascertained that with a resistance of 9,110 ohms in the resistance box the average value of x on the Wheatstone bridge for the water in the vessel after being rinsed and before placing the roots therein was approximately 6.0, rarely varying 1 cm. either way. Considering that figure, then, as the basis or the starting point for the exosmosis, and subtracting it from the different values found for the renewed, and from only the final value obtained for the unrenewed distilled water, we get the figures in the last column of table VIII.

The plan of the experiment with respect to renewal of the distilled water and the time of readings, the values of the individual readings, and the comparative amounts which represent the total exosmosis of the electrolytes under the various conditions of the experiment are all given in table VIII. The numbers given are the values of x on the Wheatstone bridge when the resistance inserted in the box was 9,110 ohms.

It is thus seen that by far the greater exosmosis was obtained in the case of those cultures in which the distilled water was renewed. Another point of interest was the reabsorp-

tion of electrolytes—as seen by the decrease in conductivity of the medium—in those cultures in which the distilled water was not renewed. The reabsorption of electrolytes has been observed to be a phenomenon characteristic of normal, healthy

TABLE VIII (Series 6)
COMPARATIVE EXOSMOSIS IN RENEWED AND UNRENEWED DISTILLED WATER

Culture no.	Water renewal	Conductivity Readings								Duration of treatment days	Total increase in conductivity
		Frequency	1st	2nd	3rd	4th	5th	6th	7th		
1	Every day	Every day	32.9	10.4	10.0	8.9	9.7	9.4	10.2	7	49.5
2	None	Every day	36.3	22.8	21.4	17.8	15.2	12.5	11.4	7	5.4
3	Every 2 days	Every 2 days	10.8	9.3	9.6	10.7	....	....	....	8	16.4
4	None	Every 2 days	25.0	14.3	13.6	11.0	....	....	....	8	5.0
5	Every 4 days	Every 4 days	12.9	15.0	16.1	16.1	....	....	....	16	36.1
6	None	Every 4 days	10.7	12.4	15.9	19.5	....	....	....	16	13.5

peas, when transferred from a full nutrient solution to distilled water, after being in the latter medium one or two days.

In order to obtain some additional information regarding the relations between the conductivity of the medium and the plants grown therein, series 7 containing 50 cultures was set up in full nutrient solution, ten Canada field pea plants to each culture. The nutrient solution was not renewed. At the end of each five-day period 5 of the cultures were taken down, the green weight of tops of the plants in each determined, and the conductivity of the solution measured; and from these results the average green weight of tops and the average conductivity of each set of 5 cultures were obtained. This was done throughout the entire period of 50 days. The results obtained are given in table IX and plotted as curves in fig. 1. In the latter the abscissa represents days, and the ordinate both specific conductivity and green weight of tops. The values given for conductivity should be multiplied by 10^{-5}

in order to get the specific conductivity values. In the case of the weights the numbers in the margin represent ten times the actual weight in grams, e.g., 40 in the margin = 4.0 grams.

From the results it is seen that both the increase in green

TABLE IX (Series 7)

GROWTH OF PLANTS AND CONDUCTIVITY OF FULL NUTRIENT MEDIUM FOR 50 DAYS

Cultures nos.	Length of period in full nutrient days	Av. green wt. of tops in each culture grams	Specific conductivity* at end of period		
			Minimum	Average	Maximum
1– 5	5	3.81	93.22	96.25	98.19
6–10	10	8.12	57.47	61.03	66.93
11–15	15	9.47	32.38	34.83	37.59
16–20	20	8.66	24.38	32.68	46.05
21–25	25	8.69	15.73	18.19	21.69
26–30	30	8.00	13.74	20.63	30.28
31–35	35	7.10	10.02	15.98	21.23
36–40	40	6.77	11.16	14.23	16.19
41–45	46	6.09	6.88	11.44	22.51
46–50	50	5.01	13.00	16.97	28.11

* The numbers in the three columns are to be multiplied by 10^{-5} in order to arrive at the specific conductivity values.

weight of tops and decrease in conductivity of the medium are most rapid and pronounced during the first 15 days. After that period both the green weight and the conductivity gradually decline, but the latter more slowly than the former. While the curves of the minimum, average, and maximum conductivity remain very close together during the first 15 days, they become more divergent after that time. The green-weight curve shows a gradual decline as the age of the plant increases, after a certain period, due to the drying of the tops and consequent loss of water. Different curves would, of course, have been obtained had the nutrient solution been renewed.

In table x and fig. 2 are seen the results of series 8, a similar experiment with distilled water, the same units being used as in the previous case. The green weight of tops increased during the first 10 days and then gradually declined to the end of the experiment. The conductivity of the water was practically the same on the 10th as it had been on the 5th day. Evidence from other experiments, however, indicates

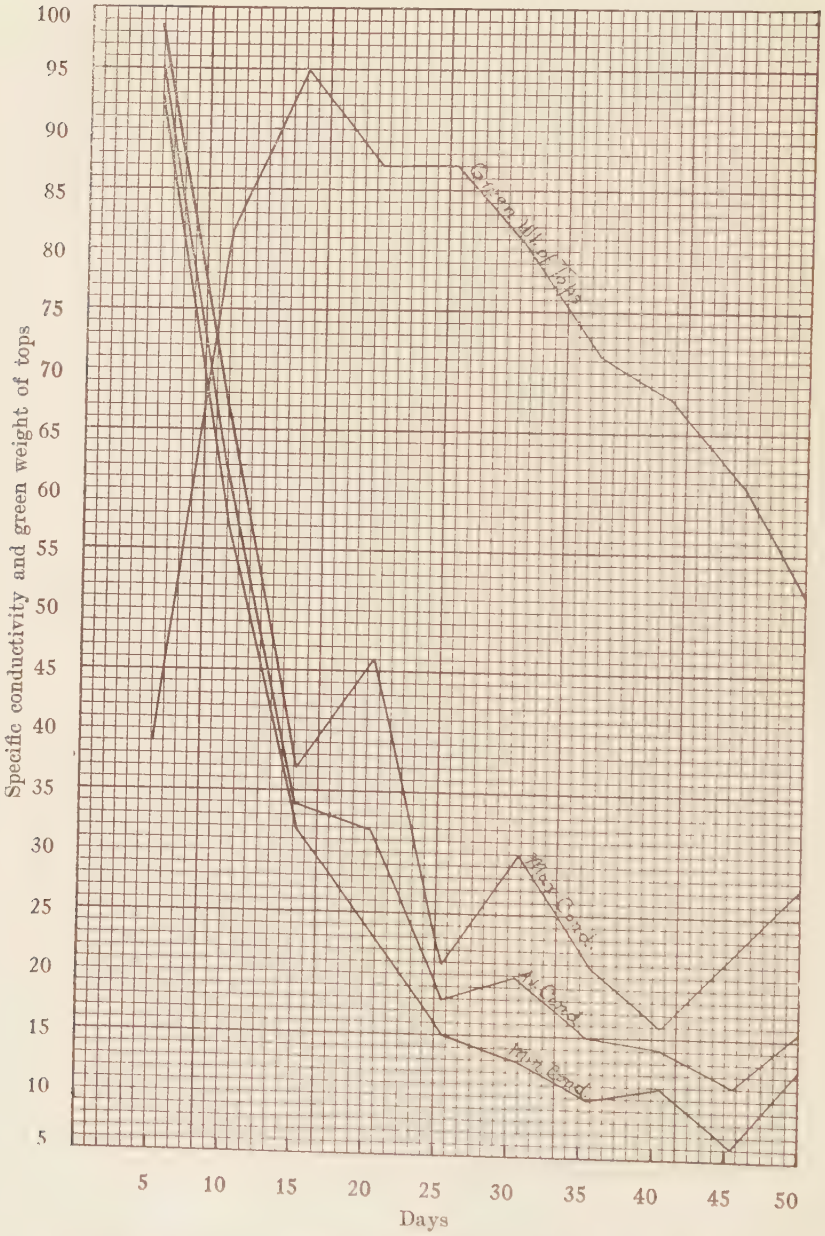

Fig. 1. The conductivity and growth curves for the full nutrient solution (Pfeffer's) in which plants were grown 50 days, the medium being unrenewed. (For complete explanation see the text.)

that in the interim the curve might have risen and fallen. After the 10th day the curve inclined with fluctuations. Here again are seen evidences that the 10-day period for seedlings in the distilled water may properly be considered a crucial one for the plants. After that time the growth declines and the conductivity increases markedly.

Suspecting that the question of injury to plants in distilled

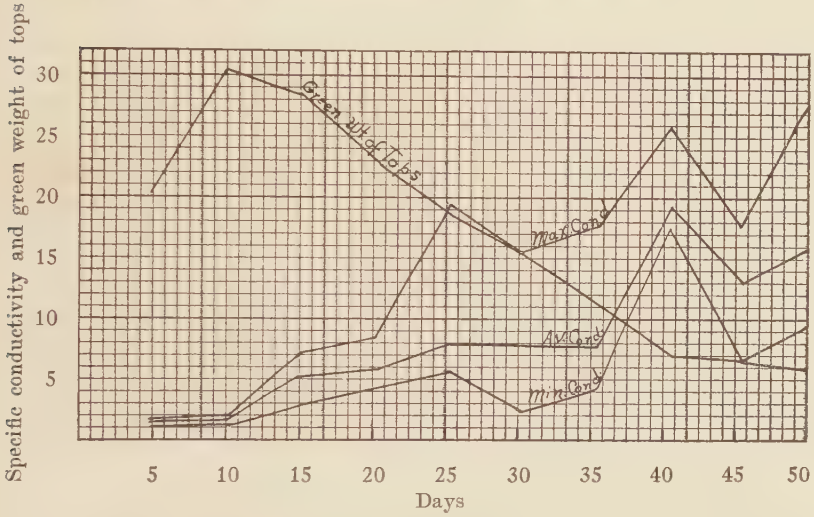

Fig. 2. The conductivity and growth curves for the unrenewed redistilled water in which pea seedlings were grown for 50 days. (For complete explanation see the text.)

water might be intimately bound up with that of lack of reserve food materials, the writer carried out an experiment bearing upon this matter. The experiment consisted, first, in placing some Canada field pea seedlings directly into redistilled water and determining the specific conductivity of the water at intervals for 20 days; and, next, in transferring some pea plants which had been grown in full nutrient solution for 1, 5, 10, 20, 30, and 40 days respectively to redistilled water, and determining the specific conductivity of the water at intervals for 20 days. The results are plotted as curves in fig. 3, the conductivity values being represented in terms of x on the Wheatstone bridge with a resistance of 9,110 ohms in the box. Four cultures of 10 plants each (except in the case

of the 20-day period in full nutrient solution in which 12 cultures were used) were grown under each of the specified conditions, and the curves represent the averages for the 4 (or 12) cultures under each condition. To determine how much

TABLE X (Series 8)

GROWTH OF PLANTS AND CONDUCTIVITY OF DISTILLED WATER MEDIUM FOR 50 DAYS

Cultures nos.	Length of period in dist. water days	Av. green wt. of tops grams	Specific conductivity* at end of period		
			Minimum	Average	Maximum
1– 5	5	2.04	1.46	1.54	1.66
6–10	10	3.07	1.01	1.34	1.81
11–15	15	2.89	3.04	5.43	7.41
16–20	20	2.34	4.34	5.92	8.63
21–25	25	1.89	5.66	8.15	19.77
26–30	30	1.55	2.60	8.26	15.82
31–35	35	1.15	4.43	7.80	17.95
36–40	40	.72	17.62	19.51	26.04
41–45	45	.68	6.87	13.32	17.95
46–50	50	.60	10.51	16.48	29.00

* The numbers in the three columns are to be multiplied by 10^{-5} in order to arrive at the specific conductivity values.

increase in conductivity was contributed by the glass tumblers in which the cultures were grown, 4 such containers filled only with redistilled water, and containing no plants, were used and the conductivity of the water determined at intervals for 20 days. It is seen that from the seedlings which had not been in full nutrient solution at all (Nos. 5–8) the highest conductivity resulted, while from those which were in the full nutrient solution longest before being placed in the distilled water (Nos. 37–40 and 33–36), the lowest conductivity was found at the end of 20 days. The other cultures at the end of 20 days were midway between the two extremes. It is also seen that, whereas the conductivity curve for Nos. 5–8 shows very little tendency to decline in the early stages, the curves for the cultures which had first been in full nutrient solution show that tendency to a considerable extent. And that tendency, as we have previously remarked, is a characteristic feature of normal plants transferred from full nutrient solution to distilled water.

Attention should be called to the difference in the character of the conductivity curves in fig. 2 and that of 5–8 in fig. 3. It

will be noted that both represent the conductivity curve of distilled water containing the roots of seedling peas. The difference mentioned no doubt finds its explanation in the different conditions under which the two series were grown (the series

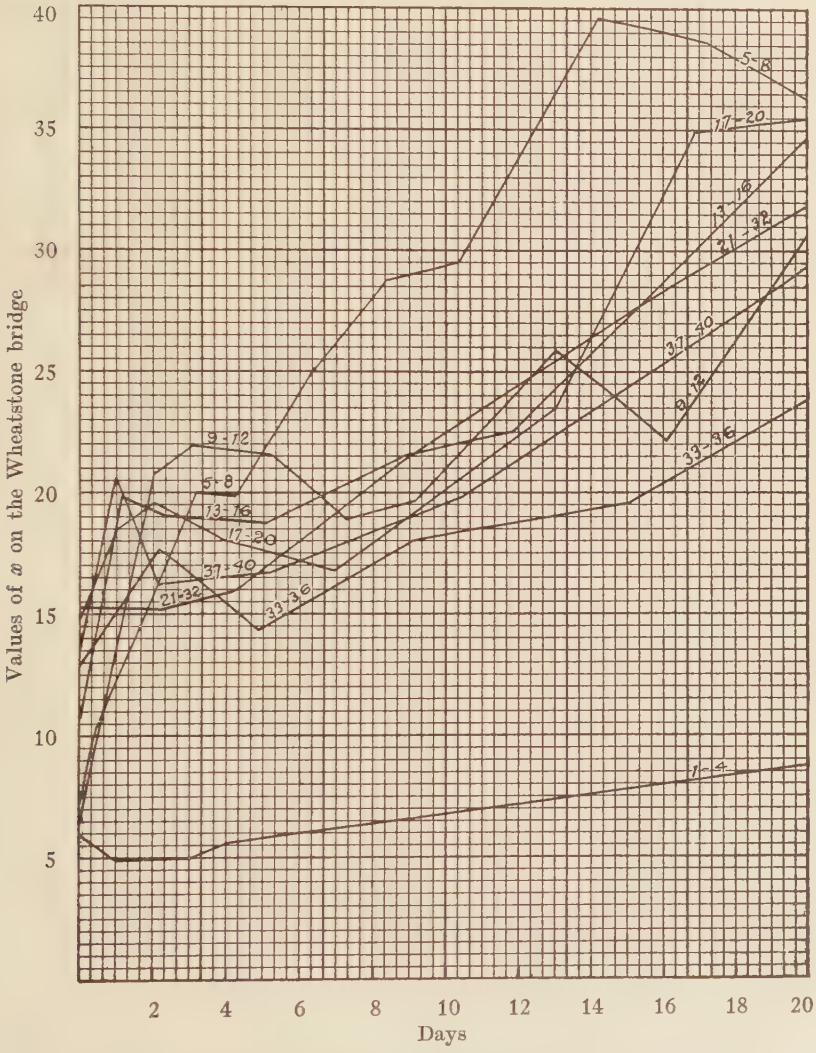

Fig. 3. The conductivity curves for cultures in distilled water 20 days—after growth in full nutrient solution for varying periods of time, as follows: Nos. 9–12, 1 day; Nos. 13–16, 5 days; Nos. 17–20, 10 days; Nos. 21–32, 20 days; Nos. 33–36, 30 days; Nos. 37–40, 40 days. Nos. 5–8 were grown only in distilled water, while Nos. 1–4 were without plants, consisting only of distilled water.

for fig. 2 being run in the fall when the seeds were fresh, and that for fig. 3 in the winter), in the vigor of the seeds, and in the difference in the units used in plotting the curves. It must be said, however, that various factors of the problem of exosmosis from the roots of plants remain as yet unknown.

The early drop in the curve of the conductivity of the controls (1–4) is an interesting feature which would seem to be explained by an adsorption of the electrolytes on the surface of the chemically clean glass tumblers.

At the end of 20 days in distilled water the roots of the plants which had not been in full nutrient at all showed marked deterioration (being badly decomposed and covered with a gelatinous coating), while the roots of those which had previously been in full nutrient solution for some time remained normal in every respect, even after 20 days in distilled water.

These results seem plainly to indicate that injury which plants sustain in distilled water is very closely related either to the lack of available nutrients in the medium or of reserve food material in the tissues. A seedling is in an exceedingly plastic state of growth. If no food materials become available the embryonic tissues which are in such an active condition of growth soon become disorganized, possibly suffering partial autolysis and becoming the prey to bacterial and fungous action. We would expect, therefore, that the larger the seeds (and hence also the supply of stored materials), the longer the seedlings could remain in distilled water before deterioration. Comparison of True's results on *Lupinus* with those here presented on *Pisum sativum* and *Vicia faba* seems to fulfill that expectation. We should also expect that the more nutrient materials the plant absorbed, the better it would be able later to withstand any deteriorating influences in the distilled water, and the experiment above noted seems to bear out that idea also.

In the light of what has been said we are led to believe that the conductivity curve of Nos. 5–8 is not a pure representation of exosmosis and that the products of bacterial and fungous action and cell decomposition account for at least a part of the conductivity. While the same condition may be

true of the other cultures to a certain extent, it no doubt plays a lesser, and real exosmosis a greater, part.

In connection with the above experiment it was thought desirable to determine whether a difference in the initial temperature of the water into which the roots were placed had any immediate or subsequent effect upon the exosmosis from the roots; plants which had been grown in full nutrient solution for 20 days were used for this purpose. Four cultures were prepared with distilled water at a temperature of 6.5°C., four at 17.2°C., and four at 35.0°C., and conductivity readings were taken after exactly one-half hour, and then at various intervals for 20 days. No attempt was made to keep the water at the initial temperatures and it therefore gradually returned to the temperature of the room. After one-half hour, when the first readings were taken, the respective temperatures were 8.9°C., 16.6°C., and 27.4°C.

The average conductivities of the water of these cultures are plotted for 20 days in fig. 4, the same units being used as in fig. 3. From these results it may be concluded that the initial differences of temperature can not be said to have exercised much, if any, effect. The results would probably have been different had the temperatures remained at the original point during the 20 days. Wächter ('05) has considered the rôle of the temperature factor in exosmosis.

VIII. Discussion and Conclusions

It is believed that the evidence furnished is sufficient to support the conclusion that pure distilled water *per se* is not toxic or injurious to plants, and that various other factors enter in to cause the deterioration noted when plants are placed in that medium.

Of course by qualifying the assertion to include *pure* distilled water only, we have thus eliminated the effect that may be produced by toxic substances in the distilled water, no matter from whence derived. The abundance of work that has been done on the toxicity of various substances to plant tissues would of course lead us to expect injurious effects if such substances were present in any quantity in the distilled

water. With that phase of the question we are therefore not much concerned at present. With a distilled water prepared as indicated, and with a specific conductivity which is approximately 2×10^{-6}, we have a water sufficiently pure for use in the consideration of other aspects of the question, and attention is directed to these.

The evidence presented has inclined us strongly to the view

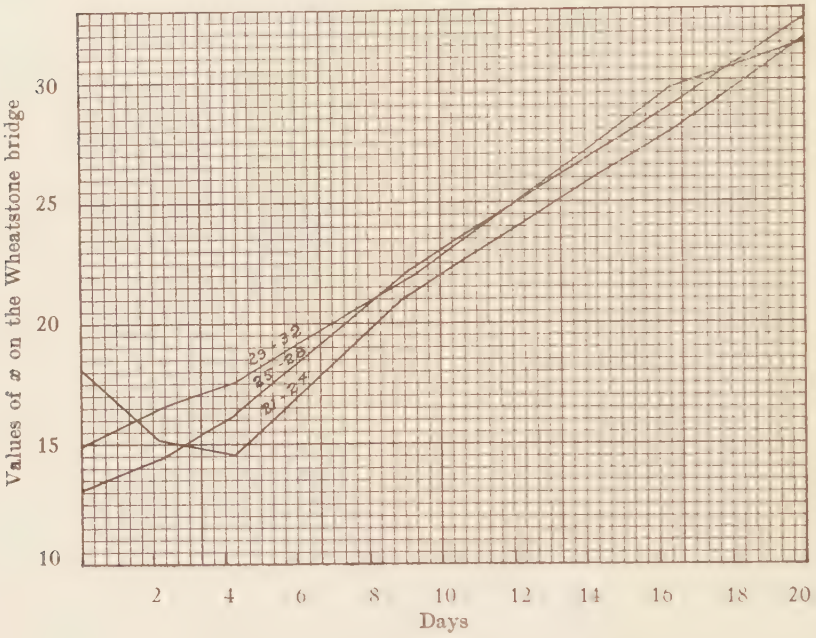

Fig. 4. The conductivity curves for cultures in distilled water 20 days—after growth in full nutrient solution for 20 days. The initial temperatures of the distilled water into which the roots were placed were as follows: Nos. 21–24, 6.5°C.; Nos. 25–28, 17.2°C.; Nos. 29–32, 35°C.

that the fundamental basis of the deterioration of plants in distilled water rests upon the food relations of such plants, but that, on the other hand, an exosmosis of food materials or nutrient salts is in no way responsible for the difficulty. It is considered that the question of the food relation plays an important rôle in the incipiency of the disorder, but that this is quickly followed by factors which have been initiated as a result of the inimical food or nutrient relation.

A plant must assuredly have food in order to thrive. The more food it has stored up in its tissues, the longer it can survive in a medium devoid of it. But because of the absence of available food it is believed that the tissues of the plant begin to become disorganized and in that condition fall a ready prey to bacterial and fungous action, which may then set in and play a very important part in the subsequent decomposition of the tissues.

While it may seem paradoxical to assert in one clause that absence of food is the fundamental basis of the injury which plants undergo in distilled water, and in the very next to say that exosmosis of nutrient salts plays no rôle, yet the results obtained have substantiated that idea. Furthermore, it is essential to consider the various other factors attendant upon these two conditions in order to arrive at the proper conclusions respecting their operation. Among such factors may be mentioned the decrease in conductivity after a short period coincident with exosmosis from normal tissues, the relation of sterilization to bacterial and fungous action, the recovery of plants under different conditions, and the numerous other questions already considered in the body of the article, all of which lend weight to the conclusions arrived at.

IX. Summary

A brief historical review is given in this paper of the views held in regard to the cause of injury to plants in distilled water.

The methods of work are outlined.

The experimental work is given and the results discussed, especially with reference to the conclusions of other workers.

A discussion is given of the results obtained in the experimental work and the conclusions derived therefrom are stated.

Some of the results obtained from the experimental work may be summarized as follows:

(a). Renewing the distilled water of the cultures every 4 days was in general beneficial, as shown by increased growth of both tops and roots. The plants were also able to survive longer in the renewed than in the unrenewed distilled water,

and continued growth better after being placed in a full nutrient solution.

(b). The period between 5 and 10 days in distilled water is a crucial one for plants; if they remain longer in this medium they are unable to recover normally or completely when subsequently placed in a full nutrient solution.

(c). By keeping the plants in distilled water a certain period before transferring to full nutrient solution the maturity of the plants is delayed.

(d). The longest period during which plants can be kept in distilled water and later recover on being placed in full nutrient solution was found to be 30–40 days. For certain dilute toxic solutions this period was much less, thus indicating that the so-called toxicity of distilled water is, if it exists at all, very slight.

(e). The lateral roots of "boundary cultures" were characteristically long and thread-like.

(f). Sterilizing the distilled water by boiling one-half hour every 4 days exercised a beneficial effect upon the growth of plants in that medium as compared with the growth of those in unsterilized distilled water.

(g). Greater total exosmosis was obtained in the renewed than in the unrenewed distilled water.

(h). Normal plants which have been grown for some time in full nutrient medium and then transferred to distilled water exhibit at first greater excretion than absorption of electrolytes. After one or two days, however, there is greater absorption than excretion and the conductivity curve declines. This condition may be maintained for a considerable period.

(i). The conductivity curve of the full nutrient solution in which plants were grown rapidly fell during the first 15 days or so; then it was more or less horizontal for a period, and finally began to incline after about 50 days. The growth curve was in general opposite in character to the conductivity curve.

(j). The conductivity of the distilled water in one series in which the roots of pea seedlings were placed was practically the same on the 10th as on the 5th day. After the 10th day

it rose considerably. The growth curve showed a rise the first ten days, then a decline.

(k). Higher conductivity in the distilled water after 20 days was caused by plants which had not previously been in full nutrient solution than by plants grown for a time in full nutrient solution before transference to distilled water. The former cultures also failed to give the decline in conductivity characteristic of normal plants transferred from full nutrient solution to distilled water.

(l). Greater deterioration of the roots in distilled water occurred if the plants had not previously been in full nutrient solution than in the case of plants which had been grown for a time in the latter medium.

(m). Initial difference of temperature of the distilled water produced no effect on the exosmosis of electrolytes.

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Explanation of Plate

PLATE 13

Figure 1.

Culture no.		Conditions of growth.
1	(2)	*Unrenewed distilled H_2O, 45 days.
2	(3)	Renewed distilled H_2O, 45 days.
3	(6)	1 day dist. H_2O, 44 days in renewed full nutr.
4	(8)	2 days dist. H_2O, 43 days in renewed full nutr.
5	(10)	5 days dist. H_2O, 40 days in renewed full nutr.
6	(14)	10 days dist. H_2O (renewed), 35 in renewed full nutr.
7	(18)	15 days dist. H_2O (renewed), 30 in renewed full nutr.
8	(22)	20 days dist. H_2O (renewed), 25 in renewed full nutr.
9	(23)	45 days in unrenewed full nutr.
10	(25)	45 days in renewed full nutr.

Figure 2.

1	(9)	5 days in unrenewed dist. H_2O, 40 days in unrenewed full nutr.
2	(10)	5 days in unrenewed dist. H_2O, 40 days in renewed full nutr.
3	(11)	10 days in unrenewed dist. H_2O, 35 days in unrenewed full nutr.
4	(12)	10 days in unrenewed dist. H_2O, 35 days in renewed full nutr.
5	(13)	10 days in renewed dist. H_2O, 35 days in unrenewed full nutr.
6	(14)	10 days in renewed dist. H_2O, 35 days in renewed full nutr.
7	(19)	20 days in unrenewed dist. H_2O, 25 days in unrenewed full nutr.
8	(20)	20 days in unrenewed dist. H_2O, 25 days in renewed full nutr.
9	(21)	20 days in renewed dist. H_2O, 25 days in unrenewed full nutr.
10	(22)	20 days in renewed dist. H_2O, 25 days in renewed full nutr.

* The numbers in parentheses correspond to the culture numbers of series 1. (See table 1.)

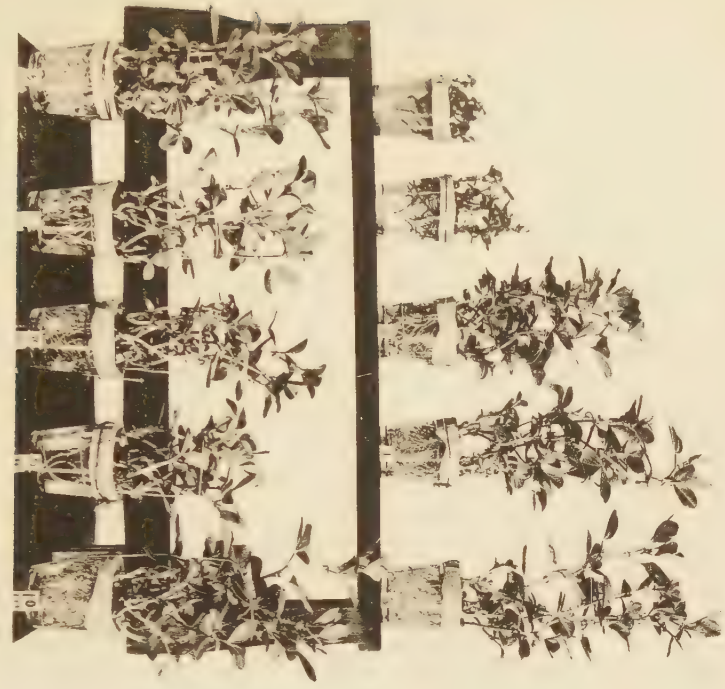

Fig. 1

Fig. 2

MERRILL—DISTILLED WATER

Explanation of Plate

PLATE 14

Figure 1.

Culture no.	Conditions of growth.
1	(2) *33 days in unrenewed dist. H_2O at time picture was taken.
2	(3) 33 days in renewed dist. H_2O at time picture was taken.
3	(6) 1 day in dist. H_2O, 32 days in renewed full nutr.
4	(8) 2 days in dist. H_2O, 31 days in renewed full nutr.
5	(10) 5 days in unrenewed dist. H_2O, 28 days in renewed full nutr.
6	(14) 10 days in renewed dist. H_2O, 23 days in renewed full nutr.
7	(18) 15 days in renewed dist. H_2O, 18 days in renewed full nutr.
8	(22) 20 days in renewed dist. H_2O, 13 days in renewed full nutr.
9	(23) 33 days in unrenewed full nutr.
10	(26) 33 days in renewed full nutr.

Figure 2.

1	(9) 5 days in unrenewed dist. H_2O, 28 days in unrenewed full nutr.
2	(10) 5 days in unrenewed dist. H_2O, 28 days in renewed full nutr.
3	(11) 10 days in unrenewed dist. H_2O, 23 days in unrenewed full nutr.
4	(12) 10 days in unrenewed dist. H_2O, 23 days in renewed full nutr.
5	(13) 10 days in renewed dist. H_2O, 23 days in unrenewed full nutr.
6	(14) 10 days in renewed dist. H_2O, 23 days in renewed full nutr.
7	(19) 20 days in unrenewed dist. H_2O, 13 days in unrenewed full nutr.
8	(20) 20 days in unrenewed dist. H_2O, 13 days in renewed full nutr.
9	(21) 20 days in renewed dist. H_2O, 13 days in unrenewed full nutr.
10	(22) 20 days in renewed dist. H_2O, 13 days in renewed full nutr.

* The numbers in parentheses correspond to the culture numbers of series 2. (See table III.)

Fig. 1

Fig. 2

MERRILL—DISTILLED WATER

Explanation of Plate

PLATE 15

Figure 1.

Culture no.		Conditions of growth.
1	(1)	*10 days in unrenewed dist. H_2O, 42 days in unrenewed full nutr.
2	(2)	10 days in unrenewed dist. H_2O, 42 days in renewed full nutr.
3	(3)	10 days in renewed dist. H_2O, 42 days in unrenewed full nutr.
4	(4)	10 days in renewed dist. H_2O, 42 days in renewed full nutr.
5	(5)	20 days in unrenewed dist. H_2O, 32 days in unrenewed full nutr.
6	(6)	20 days in unrenewed dist. H_2O, 32 days in renewed full nutr.
7	(7)	20 days in renewed dist. H_2O, 32 days in unrenewed full nutr.
8	(8)	20 days in renewed dist. H_2O, 32 days in renewed full nutr.
9	(9)	30 days in unrenewed dist. H_2O, 22 days in unrenewed full nutr.
10	(10)	30 days in unrenewed dist. H_2O, 22 days in renewed full nutr.
11	(11)	30 days in renewed dist. H_2O, 22 days in unrenewed full nutr.
12	(12)	30 days in renewed dist. H_2O, 22 days in renewed full nutr.
13	(13)	40 days in unrenewed dist. H_2O, 12 days in unrenewed full nutr.
14	(15)	40 days in renewed dist. H_2O, 12 days in unrenewed full nutr.

Figure 2.

1	(1)	†32 days in unrenewed dist. H_2O.
2	(2)	32 days in renewed dist. H_2O.
3	(20)	32 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$.
4	(22)	1 day in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 31 days unrenewed full nutr.
5	(23)	2 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 30 days unnewed full nutr.
6	(24)	4 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 28 days unnewed full nutr.
7	(25)	8 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 24 days unrenewed full nutr.
8	(26)	12 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 20 days unnewed full nutr.
9	(27)	16 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 16 days unrenewed full nutr.
10	(28)	20 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 12 days unrenewed full nutr.
11	(29)	32 days in unrenewed full nutrient solution.

*The numbers in parentheses correspond to the culture numbers of series 3. (See table v.)

†The numbers in parentheses correspond to the culture numbers of series 4. (See table vi.)

Fig. 1

Fig. 2

Explanation of Plate

PLATE 16

Figure 1.

Culture no.	Conditions of growth.
1	(1) *30 days in unrenewed distilled H_2O.
2	(2) 30 days in renewed distilled H_2O.
3	(3) 30 days in distilled H_2O, sterilized every four days.
4	(4) 30 days in distilled H_2O, sterilized every four days.
5	(5) 30 days in unrenewed full nutrient solution.
6	(6) 30 days in renewed full nutrient solution.

Figure 2.

Showing the method used for seed germination.

*The numbers in parentheses also correspond to the culture numbers of series 5. (See table VII.)

Fig. 1

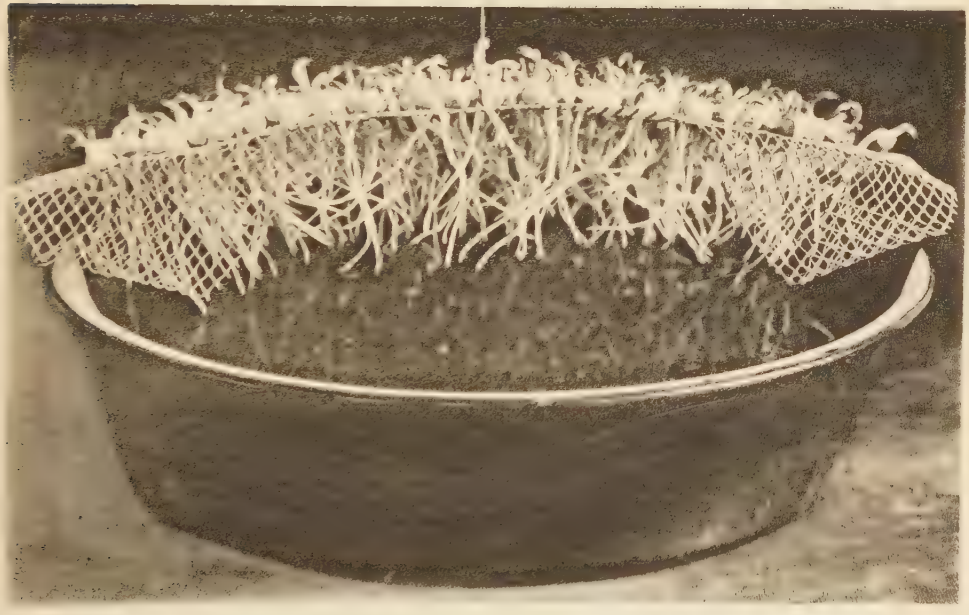

Fig. 2

MERRILL—DISTILLED WATER

ELECTROLYTIC DETERMINATION OF EXOSMOSIS FROM THE ROOTS OF PLANTS SUBJECTED TO THE ACTION OF VARIOUS AGENTS

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I. Introduction

In a previous paper the writer ('15) gave some results showing the exosmosis curves when normal growing plants are taken from a full nutrient medium and placed in redistilled water. Those results and the data herewith given show that exosmosis of electrolytes is a constant feature associated with the transfer of normal growing plants from a full nutrient solution to distilled water. In the paper above mentioned evidence was introduced indicating that such exosmosis was not a causal injury but that it was simply a concomitant condition or incidental effect and had but an indirect relation to the inimical condition of the plant in the distilled water. For convenience we might designate the agency or agencies causing such exosmosis as *passive* in their effects.

In this paper are given results on exosmosis in terms of the electrolytic conductivity of the medium when such excretion is caused, or at least is accelerated, by various factors or agencies which we may designate as *active* in their effects. Accordingly, plants have been treated by injurious agents or subjected to conditions of different kinds and the comparative effects on the exosmosis from the roots have been noted. By determining the conductivity of the medium at various intervals subsequent to the treatment, data have been secured for plotting the exosmosis curves shown in this paper. It has also been the aim to determine in each case the approximate boundary between the normal and the abnormal exosmosis by varying either the duration of application or the concentration of the substance applied, or both. Hence in most cases there will be found the two extremes with any given substance—at the upper end of the scale the curve of excessive exosmosis due to

cytolysis or death of the cells (though it should be noted here that excessive exosmosis from the roots may result even when those tissues are in an apparently normal condition), and at the lower end of the scale the curve of slight exosmosis that is in the region of the normal curve of exosmosis for untreated plants placed from full nutrient solution into distilled water. Between these two extremes lie various gradations depending on conditions.

II. Historical Review

The work that has been done on the problem of excretions from the roots of plants is very interesting from several standpoints and has been considered by various workers to be of great practical importance. Nearly a century ago De Candolle ('32) advocated a theory of crop rotation on the basis of root excretions in which he claimed that certain plants excreted from their roots substances which are harmful to succeeding crops of closely related plants, but not so to plants less closely related. This theory was based partly on his own observations and partly on the statements of earlier workers.

At De Candolle's suggestion Macaire ('32) performed some experimental work pertaining to root excretions. He took plants from the soil, washed the roots carefully, and placed them in rain water. After several days, during which the water was frequently changed, the water was yellow and had odor, taste, and chemical reactions indicative of contained exuded materials. By placing one part of the roots of a plant in a vessel of pure water and another part in a second vessel containing a solution of lead acetate and later finding the salt in the pure water, he concluded that a plant can excrete a poison which it has absorbed. The results of Macaire's experiments with water cultures led him to favor the theory of crop rotation on the basis of the excretions from the roots of plants, as advanced by De Candolle.

Braconnot ('39) repeated many of Macaire's experiments but was unable to convince himself that plants excrete toxic substances from their roots, and hence he did not look with favor upon De Candolle's theory. Braconnot believed that

capillary action played a rôle in Macaire's experiments whereby he obtained an excretion of lead acetate into distilled water, as noted above. Boussingault ('45) considered that under ordinary conditions radicular excretion is doubtful, and that any excretion from the roots in water is caused by disease. He also advanced various arguments opposing De Candolle's theory.

Gyde ('47) grew various agricultural plants in soil for a time and then, after carefully washing the roots, placed them in pure water. After 3–17 days, during which the plants continued in good condition for the most part, the water was evaporated. The finding of a residue of yellowish or brown matter, part organic and part inorganic, caused him to conclude that plants excrete both organic and inorganic substances in minute quantities, similar in composition to the sap. But he denied that root excretions have any injurious effect upon plants later grown in the same medium.

An examination of the literature on the subject of root excretions reveals the tendency among the workers of the particular period at which we have now arrived in our review, to pay more attention to the morphological and chemical aspects of root excretions, and perhaps not so much to the purely agricultural phases. Hence we find from this period on, considerable emphasis laid on the structure of the root and a more detailed account given regarding the chemical nature of the substances excreted from the roots, even though the experimental methods were somewhat crude in most cases. Furthermore, it should be said that opinion was divided on the question of whether or not there is an actual excretion from the roots.

Among those whose influence was felt in the development of the chemical aspects of the subject at this time Liebig should probably be mentioned first. In the American edition of his work ('41, p. 195) occurs the following statement: "It is evident that plants, also, by producing carbonic acid during their decay, and by means of the acids which exude from their roots in the living state, contribute no less powerfully to destroy the coherence of rocks." An appended note by Dr. Webster in the

same work ('41, p. 411) says that other chemists were unable to obtain results similar to those of Macaire. If they did, they were inclined to ascribe them to injury of the roots examined.

Various workers were thus attacking different phases of the problem. Chatin ('47) mentioned the excretions from roots and especially considered the elimination of toxic substances by them. Link ('48) held that the slimy drops found on root tips should not be considered as actual excretion inasmuch as they arise from the cast-off cap cells of the root. Garreau and Brauwers ('58) maintained a similar view in regard to the gummy, nitrogenous substance they found given off by the roots to the water in which they were placed. The observations of Liebig ('58) concerning the dissolving action of roots on limestone were later substantiated by the experimental work of Sachs ('60), which has been so much referred to since that time. Of the two possible explanations Sachs advanced —excretion of carbonic acid by the roots, and the liberation of acids by the decomposition of the cell walls of the roots—he inclined to favor the latter as being the best explanation for the marble etchings caused by the roots in his experiments. In his extensive series of experiments, Knop ('60, '61, '62) studied, among other things, the character and amount of root excretions from certain plants placed in distilled water, and the conditions governing the same. His analyses indicated that, in addition to other substances in small amounts, potassium, calcium, phosphoric acid, and some organic matter were excreted. The studies of Cauvet ('61) resulted in his declaring that physiologically sound roots do not excrete any substances, toxic or otherwise, and that all theories based on the ideas of root excretion advanced by De Candolle and Macaire were necessarily false. Sachs ('65) made further contributions along his line of work indicated above, while Liebig ('65) says:

> "Wir haben allen Grund zu glauben, dass diese Absonderung an der ganzen Oberfläche stattfindet, wir beobachten sie nicht nur am Stamme, sondern auch an den kleinsten Zweigen, und wir müssen daraus schliessen, dass dieser Excretionsprocess auch an den Wurzeln vor sich geht. . . . . Eine Ausscheidung von Excrementen kann demnach bei den Pflanzen

nicht geleugnet werden, wiewohl es möglich ist, dass sie nicht bei allen Pflanzen in gleichem Grade stattfindet."

Molisch ('87) branched out in a new direction as regards the subject of root excretions; he held that such excretions exercise an influence on organic bodies in the soil which is even more important than that exercised upon the inorganic constituents of the same, for he considered the latter merely a dissolving action but the former a real chemical transformation. His main work along this line pertained to a study of the ferments in the root excretions, and their reactions and properties. Johnson ('90), after considering Gyde's results above noted, says that "we may well doubt whether agricultural plants in the healthy state excrete any solid or liquid matters whatever from their roots," but that "under certain circumstances, small quantities of soluble salts or free acids may indeed *diffuse* out of the root-cells into the water of the soil. This is, however, no physiological action, but a purely physical process." Goebel ('93) found that after the roots of Hordeum and Lepidium plants had been in distilled water for six days the medium gave the reaction for formic acid.

We thus see that the early work on root excretions was characterized by contradictions and uncertainties. While the nature of the more recent work has been more exact and comprehensive, the subject, as we shall see, is still beclouded by a considerable degree of confusion.

A classic piece of experimental work was undertaken by Czapek ('96, '96[a]) to determine the exact chemical nature of the excreted substances from roots. In his report ('96[a]) he discussed the earlier work, especially with regard to the relation between excretion from injured cells and actual exosmosis. In his experimental work he found that root excretions are composed of soluble substances, partly organic and partly inorganic. Of the inorganic, he identified K, Ca, Mg, HCl, H_2SO_4, and H_3PO_4, only the first and last mentioned—in the form of the primary potassium phosphate—being excreted in any quantity. Of the organic substances he identified carbonic acid and also formic acid, the latter in the form of its potassium salt; oxalic acid was also isolated as a primary

potassium salt. Czapek believes the reddening of litmus paper by root excretions to be due ordinarily to the acid reaction of monopotassium phosphate, but in the case of hyacinth roots to the primary oxalate. The corrosion of marble he attributed to the dissolving effect of carbonic acid. While considering as possible the results obtained by Molisch ('87), who claimed that diastatic ferments were normally present in the root excretions, Czapek's own work in repetition of Molisch's experiments offered only negative results.

Prianischnikov ('04) performed some experimental work dealing with the action of organic acids on phosphates. It will be remembered that because the roots did not attack aluminum phosphate Czapek concluded that organic acids were not excreted by them, inasmuch as this substance is soluble in certain organic acids. Prianischnikov found that phosphates derived from different sources were utilized by various plants but in different degree, and he suggested that this might be correlated with a different amount of CO_2 excretion, in which case the presence of organic acids would not be necessary.

Kunze ('06) found that free mineral acids are not excreted from the roots of higher plants and concluded that any acidity in the excretions is probably not due to the presence of acid salts of mineral acids, but to excreted organic acids. These, however, were present in such minute amounts as to be below the sensitiveness of litmus. He held that a greater effect is produced on the soil by fungi than by the roots of the higher plants. Lemmermann ('07) held views similar to those of Kunze.

Stoklasa and Ernest ('08) disagree with the findings of both Czapek and Kunze. No potassium or phosphoric acid were ever found as a result of their determinations, and they maintain that in the economy of the plant the excretion of such useful or necessary substances is unthinkable. Only CO_2 was found to be excreted under conditions of normal aerobic respiration of the root system; no other free inorganic or organic acids were detected. In aerobic respiration of the root system, they believe the organic acids in the living cells would be split up to give CO_2 and H_2, the latter then being oxidized to H_2O.

They determined the amount of CO_2 excretion per gram dry weight of roots of wheat, oats, rye, and barley. The amount varied for the different plants but a correlation was found between the amounts of P_2O_5, K, and Na contained in the dry roots of plants grown on gneiss and basalt and the amount of CO_2 excreted.

We now come to the work of various soil investigators whose results have again focused attention during the past decade upon De Candolle's original theory. The essential features of this work have become so well known that for our purpose it is not necessary to do much more than merely mention it here. Though not considering directly the phases of the subject with which we are dealing, yet the much-discussed paper by Whitney and Cameron ('03) is historically important and bears an intimate relation to the later work of the investigators in the Bureau of Soils of the U. S. Department of Agriculture, the results of which led to the so-called toxic-excretion theory. Among the workers most prominently connected with the early studies along this line may be mentioned Livingston, Britton, and Reid ('05); Livingston, Jensen, Breazeale, Pember, and Skinner ('07); Schreiner and Reed ('07); Schreiner, Reed, and Skinner ('07); Schreiner and Reed ('07[a]); and others. As is well known, opinion is much divided on the various phases of this subject, however. Among those opposing the ideas or theories advanced along this line by the investigators named above should be mentioned Hopkins ('10); Hall, Brenchley, and Underwood ('14); and others.

That the question is one upon which investigations are still being pursued is shown by the publications from various quarters. As recent examples of these the work of Molliard ('13) and Prianischnikov ('14) may be cited. The former found that peas grown in water cultures in which previous crops of peas had grown produced a smaller growth than the original crops. This he attributed to the excretion of toxic substances in the medium by the earlier plants. The latter, from his own experimental work and from the results observed by him at the Rothamsted Experiment Station, is inclined to believe that the hypothesis of root excretion is not sufficiently demon-

strated. He says that other factors, as, for example, the physical nature of the soil, decomposition of roots, change in reaction of soil, etc., might be supposed to accomplish the same results as toxic excretions from the roots. In pure distilled water he found no decrease in either the size or quality of the crops of the second and third plantings, either where wheat followed wheat or where wheat followed oats. Experiments in sand, however, showed great decrease in the amount of the harvest of the second and third crops, but this, he believes, might be explained by the operation of the above-named factors.

So much for root excretions; we now come to a general consideration of exosmosis from living cells, both under natural conditions and under treatment of different kinds. While a great deal of attention has been given in the past to the intake, or endosmosis, of substances by the cell from its surrounding medium, comparatively little has been done on the opposite effect—the outgo, or exosmosis, of substances from the cell. It should be said, however, that the latter process, both in extent and in importance, is no doubt of much less significance in the plant's economy than the former.

Sachs ('60[a]) referred to the exosmosis of soluble material from germinating seeds when they remain for some time in distilled water. Knop ('64), in his studies on the absorption of salts by healthy seeds, also determined the quantities of the different salts which pass out of the seeds during the time they are swelling in distilled water. He found that both organic and inorganic substances were excreted. Hofmeister ('67) ascertained that when fresh pieces of sugar-containing plants were placed in water, no sugar passed out of the tissues into the medium. The much-cited experiments of De Vries ('71) showed that pieces of red beet placed in water for 15 days gave no trace of sugar or of colored material to the water during that time. In a NaCl solution of sufficient concentration, however, he obtained an exosmosis of both sugar and colored material. Turnips, beets, and the seedling roots of wheat, barley, and corn were used in the experiments of Boussingault ('74) but from none of them did he detect any

exosmosis of sugar into the water in which they were placed. Pfeffer ('76, '77) and Detmer ('79) also confirmed the results above noted regarding the absence of sugar in the water in which roots or other plant parts had been exposed for some time. Wilson ('81) found that in some cases (*Dionaea* and *Drosera*) the excretions may be influenced by external factors, e. g., partly by irritation caused by nitrogenous substances and partly by osmotic action. In general, he believed that the excretion of nectar is caused by the osmotic action of a fluid on the surface of the nectary. Pfeffer ('86) studied the effects of various organic acids (citric, picric, and tannic) and some inorganic compounds in causing the exosmosis of absorbed methylene blue from *Lemna, Trianea, Azolla,* and *Elodea.*

Wächter ('05) obtained considerable exosmosis of sugar, especially in the case of *Allium Cepa*; he found, however, that salts like NaCl and KCl tended to inhibit this exosmosis. He also investigated the effect of ether on this phenomenon. While he obtained greater exosmosis of sugar the first two days in a solution of ether alone than in one of ether and KCl, he attributed this increase to leaching from cells killed as a result of contact with ether, and believed that the ether itself has no effect on the actual process of exosmosis.

Lepeschkin ('06), from his experimental work on sporangia of *Pilobolus,* concluded that the exosmosis of water was due to an alteration of the plasma membrane caused by the anesthetics he used, provided the amounts employed were sufficient to be toxic. Small amounts of ether and chloroform, on the other hand, were found to decrease the exudation of water, and he believed this to be due to a decrease in permeability of the plasma membrane.

An interesting line of investigation was undertaken by Czapek ('10, '10[a], '10[b], '11) a few years ago to determine the surface tension relations of the plasma membrane. That work is especially pertinent to our discussion here because of the prominent part exosmosis played in his experiments. He used for the most part species of *Echeveria, Spirogyra,* and *Saxifraga,* in the cells of which is found a tannoid substance,

anthocyan, which is precipitated by caffein, giving a loose compound of tannin and caffein, called a "myelin-formation." Ammonia also gives this precipitate even in a solution as dilute as 1–15,000. Czapek investigated the effect produced by the application of a great variety of organic compounds and some inorganic acids in varying dilutions and for different periods of time, and determined the concentration at which exosmosis just occurred, i. e., the critical point. At the higher concentrations exosmosis of the tannoid substance readily occurred, as shown by the absence of the "myelin-formation" when caffein or ammonia was subsequently added. At the lower concentrations exosmosis did not occur and a precipitate was obtained, while at the critical point the precipitate was barely visible and usually in the form of fine particles.

By the use of his "capillar-manometer," Czapek was able to measure the surface tension exerted by the various concentrations, and found that, considering the surface tension of water as unity, that of the critical concentrations was approximately .68 in most cases. This lowering of the surface tension he considered as essentially a physical phenomenon which is intimately connected with the osmotic activities of the plasma membrane and is to be differentiated from the toxic action of injurious substances, e. g., anesthetics, whose action is chemical in large part, since even in very dilute solutions these caused marked exosmosis. Czapek used both aqueous and colloidal solutions and found that in general the critical concentrations had a surface tension of .68 in terms of water as unity. Inversely, he therefore concluded that the surface tension of the plasma membrane was also approximately .68 for the plant cells investigated. In his study of acids he found results coincident with those of Kahlenberg and True ('96) in that N/6400 was the critical concentration for exosmosis of the tannin bodies, just as those workers had found it to be the critical concentration for growth of Lupinus seedlings in solution culture.

In his later experimental work Lepeschkin ('11) obtained additional evidence tending to confirm and add to his previous results, as mentioned above. Thus he found that aniline dyes

penetrated cells of *Spirogyra* more slowly in the presence of one per cent chloroform than when the anesthetic was not used. If the cells were killed by the narcotic the rate was the same as for normal cells. He also used *Tradescantia discolor* and by the plasmolytic method found that the permeability to KNO_3 decreased during narcosis. This he explained on the assumption that the anesthetics (chloroform and ether) accumulated in the disperse phase of the plasma membrane which thereby leads to a hindrance of the solubility of KNO_3 and aniline dyes in the same. He considered that his results therefore showed that Nathansohn's hypothesis regarding the mosaic structure of the plasma membrane is not correct.

Another important piece of work dealing with the phenomenon of exosmosis from living tissue is that accomplished by Lillie ('09, '10, '11, '12, '12[a], '13, '13[a], '13[b]) and discussed at length in his various papers. Among other things he worked on the larvae of *Arenicola* and the eggs of *Arbacia,* each of which contains a pigment, and found that on placing them in NaCl or KCl solution (.55m) isotonic with sea-water, there was a rapid exosmosis of the contained pigment into the surrounding medium. When, however, the organisms were placed in the salt solutions to which had previously been added in a certain concentration any one of several anesthetics belonging to various classes (alcohols, esters, hydrocarbons, and miscellaneous compounds) a checking or possibly a complete prevention of exosmosis resulted. In general, all the anesthetics tried gave cytolysis in strong concentrations and therefore a rapid exosmosis of the pigment, while in weaker concentrations they showed a definite protective or anticytolytic action against the salt solution when used in conjunction with it. Lillie finds the explanation of the observed phenomenon in the relations of the plasma membrane, the salt solutions used having a permeability-increasing action which is offset or prevented by the temporary alteration of the membrane as the result of the action of the anesthetic. The alteration, he believes, is accompanied by an increase in the volume of the lipoid particles of the membrane.

In connection with the general subject of exosmosis it might

be well briefly to mention the results obtained by some of the earlier investigators working on the products excreted by the leaves of plants. De Saussure (1804) found that leaves immersed in distilled water soon lose a considerable amount of substance, composed for the most part of alkaline salts. Treviranus ('38) mentioned the results of various workers who studied the incrustation of minerals on the surface of leaves and found it to consist of calcium and silicon salts, especially of calcium carbonate. Gaudichaud ('48) and Payen ('48) both found that there is an alkaline excretion on certain parts of the leaves of some plants, yet they disagreed as to the extent of this phenomenon in nature. Sachs ('62) ascertained that drops of water on the leaves of certain plants soon become alkaline, which he considered to be the result of an outward diffusion of alkaline salts in the leaf. Volkens ('84) studied the deposit of calcium carbonate found on the leaves of various plants. Dandeno ('02) made a comprehensive study of the different phases of the subject. Among other things, he determined that the alkaline substances extracted from leaves by distilled water are largely potassium and calcium carbonates and probably potassium oxalate. He further found that the residue from the evaporation of dew drops, guttation drops, and of water used in drenching the leaves is practically the same, and is similar to the calcareous deposit found upon the leaves of certain plants. The above investigations may therefore be considered as tending to substantiate the idea of exosmosis from leaves.

III. Methods of Experimentation

The methods used for the electrolytic determination of exosmosis were the same as those described in the writer's paper referred to above. In that contribution (Merrill, '15) some of the curves were plotted on the basis of the specific conductivity. In the present paper, however, all curves are plotted on the basis of the values of x on the Wheatstone bridge when the resistance in the box is 9,110 ohms; as these values increase the specific conductivity also increases. In order to have a basis of comparison between the values of x

and the specific conductivity, the corresponding values of the latter for the values of x at 5, 10, 25, 50, 75, and 85 are given herewith:

Values of x on Wheatstone bridge for resistance of 9,110 ohms.	Corresponding values in terms of specific conductivity (to be multiplied by 10^{-5})
5	.23
10	.49
25	1.49
50	4.48
75	13.46
85	25.43

It is also advisable to have the conductivity values represented in terms of the concentration of some salt. The following are the values of the specific conductivity of NaCl solutions at 25° C. which had been determined by the writer for the concentrations indicated:

Concentration of NaCl	Specific conductivity (to be multiplied by 10^{-6})
N/16	686.13
N/32	353.94
N/64	181.93
N/128	93.25
N/256	47.79
N/512	24.54
N/1024	12.60

The correction for the specific conductivity of the water itself is not considered in the above values. Neither is that correction applied in any of the work here reported, since it is always a constant factor and only relative values are desired for the most part.

Plants of *Pisum sativum* were used. For the method of growing the seedlings, and other manipulations, see the writer's paper referred to (Merrill, '15). The plants were grown in full nutrient solution until a vigorous or well-developed condition was attained and then they were transferred to redistilled water[1] after rinsing the roots carefully and thoroughly in once-distilled water. Ten plants were grown in each culture. The treatment was always given when the plants were either in distilled water or in the solution, the effects of which on the plants were being studied. In all cases where the read-

[1] Hereafter, throughout this paper, whenever "distilled water" is referred to it will be understood to mean redistilled water with a specific conductivity of approximately 2×10^{-6}. If the ordinary distilled water is referred to, it will be specially designated as "once-distilled water" or some such distinguishing term.

ings were made in the distilled water, the resistance in the resistance box was 9,110 ohms. In some media other resistances were used; in such cases the values are given only in tables, and in terms of specific conductivity.

TABLE I

EFFECTS OF VARIOUSLY TREATED PLANTS ON THE DISTILLED WATER MEDIUM AS SHOWN BY GROWTH OF SECOND CROP

Culture no.	Kind and duration of treatment	Green wt. of tops of 2nd crop* grams
1 and 2	Controls—no treatment; full nutrient to dist. H_2O	2.90
3	Plant tops packed in ice 19 hrs.; dist. H_2O unchanged	2.80
4	Plant tops packed in ice 19 hrs.; dist. H_2O changed	
5	In gas incubator at 50°C., 3.5 hrs.	1.60
6	In gas incubator at 50°C., 3.5 hrs.	2.55
7 and 8	Inoculated with *Ascochyta Pisi*	2.80
9	Illum. gas under bell jar, 6 hrs.; dist. H_2O unchanged	2.75
10	Illum. gas under bell jar, 6 hrs.; dist. H_2O changed	2.25
11 and 12	N/1 $MgCl_2$ in full nutrient as the solvent, 7 hrs.	5.20
13 and 14	.5% H_2SO_4 in full nutrient as the solvent, 7 hrs.	2.30
15 and 16	1% KOH in full nutrient as the solvent, 7 hrs.	8.45
17 and 18	Plants grown throughout in dist. H_2O; replaced by fresh seedlings in the unrenewed dist. H_2O	2.55
19 and 20	Same as Nos. 17 and 18, except that second crop was horse beans	6.85
21 and 22	Canada field peas in fresh dist. H_2O; no second crop	2.82
23 and 24	Horse beans in fresh dist. H_2O; no second crop	8.15

*The 2nd crop was 27 days old at time of weighing.

IV. Preliminary Experiments

In order to determine in a preliminary way whether the exosmosis from the roots of plants seriously affected by injurious agencies was sufficient to noticeably influence a new crop of seedlings in that medium (distilled water plus the excreted substances) as compared with control cultures in pure distilled water, the following series was set up. Canada field pea seedlings were grown in full nutrient solution until they were 15 days old, at which time they were about 8 inches high, and were green, vigorous, healthy, and in good condition. They were then treated in accordance with the plan given in table I. In some cases, depending on the nature of the agent applied, the treatment was given after the plants had been transferred to distilled water. This was the case with Nos. 3, 4, 5, 6, 9 and 10. Cultures 7, 8, and 11–16 were treated while still in the

full nutrient medium, after which they were transferred to distilled water. In all instances, however, the roots were carefully rinsed before being placed in the water.[1]

To determine if any impurities had contaminated the distilled water in the cultures treated with ice, the distilled water in No. 4 was renewed after the operation. The resulting crop, however, was practically the same in cultures 3 and 4 and hence it may be considered that no plant food had entered from the ice. The distilled water was renewed in No. 10 a few hours after the treatment. The result of so doing was to discard the plant foods already excreted during, and immediately after, the treatment. This fact was evident from the better growth of the plants in No. 9 as compared with those in No. 10. Later work also showed that exosmosis caused by treatment with illuminating gas and other agents is comparatively rapid and immediate.

After the treatment the plants remained in the distilled water for 5–6 days, after which they were discarded. The distilled water level was then raised to the original height by adding fresh distilled water, and into this medium fresh Canada field pea seedlings were placed and the resulting growth determined. Cultures Nos. 17–24 are given in table 1 for comparison. After pea seedlings had been grown for 21 days in the unrenewed distilled water of cultures 17–20, the original plants were discarded and fresh seedlings of peas and horse beans were placed in the same distilled water. For comparison, cultures of these plants (Nos. 21–24) were set up at the same time in fresh distilled water.

Returning now to the effects of the treatments on the plants and noting the results given in table 1, we see marked differences evident. Neither the ice nor the inoculation with *Ascochyta Pisi*[2] produced any effect either on the plants or on the excretions from their roots, and hence these cultures are sim-

[1] The usual method of rinsing throughout this work was as follows: The solution to be discarded was thrown out, the tumbler filled twice with once-distilled water (the roots replaced and the whole thoroughly shaken each time), and then distilled water (redistilled) was added, the roots replaced, and the readings taken.

[2] Cultures of *Ascochyta Pisi* were kindly supplied the writer by Dr. R. E. Vaughan.

ilar and comparable to the untreated controls. Marked injury resulted in the case of the heat, illuminating gas, $MgCl_2$, and H_2SO_4, all in characteristic manner. The injury from KOH was rather slow in manifesting itself, but the coloration of the roots was a noticeable feature. An interesting condition to be noted here, which holds true also in the later experiments, is in regard to the effect of the heat and the illuminating gas. It should be borne in mind that during these treatments the roots remained in water. The tops only were affected and died; the roots remained white, turgid, and normal in appearance even though the exosmosis from them had been excessive, thus indicating a transfer of some electrolytes from the tops and down into the medium through the roots. Later experiments also substantiated the fact that abundant exosmosis sometimes occurs from roots which remain normal in appearance. The other agents ($MgCl_2$, H_2SO_4, and KOH) caused more or less injury to both tops and roots. The exosmosis of nutrients into the water from the affected plants is evident by the greater growth of fresh pea seedlings placed in such water as compared with the controls. Both peas and horse beans grew somewhat better in fresh distilled water than in distilled water in which pea seedlings had already been grown for 21 days.

Further preliminary experiments along this line gave similar results. Thus in another series, vigorous, thrifty plants of Canada field peas grown 10 days in full nutrient solution were transferred, after rinsing the roots, to distilled water, some being untreated and others treated. The treatment consisted in placing some of the cultures in an atmosphere of illuminating gas for 3 and 6 hours, and others in a gas-heated oven for 1 and 2 hours where it was not aimed to keep the temperature constant. For those cultures in the oven 1 hour the temperature at the outset was 53° C. and at the end 33° C., while for those remaining in the oven 2 hours the initial temperature was 60° C. and the final 33° C. The conductivity of the water was measured soon after the plants were placed in it but before the treatment, and again 5 days after treatment,

at which time the plants were discarded and a fresh lot of pea seedlings substituted.

The average reading (value of x) of the water for the 4 untreated controls at the beginning was 14.6 on the Wheatstone bridge, and with the same resistance in the box (9,110 ohms) it was 12.6 after 5 days. For the 4 cultures treated with illuminating gas (2 cultures for 3 hours and 2 cultures for 6 hours, the resultant effect being approximately the same for the two periods of exposure) the average initial reading was 17.0 for a resistance of 9,110 ohms, and at the end of 5 days it was 43.7 for a resistance of 1,000 ohms. In this case the increase in terms of specific conductivity was from 9.2×10^{-6} to 317.3×10^{-6}.

In the 4 cultures placed in the oven at the temperature designated there was no marked difference as regards variation in conductivity of the medium. The average initial reading was 17.2 and at the end (after 5 days) it was 12.4, the resistance in the box being 9,110 ohms in both readings. The rather high initial readings in the above cases are due to the fact that it was some hours after the roots were placed in the water before the readings were taken. In later work it was found to be advantageous to have the interval between placing the roots in the water and taking the first reading reduced to exactly one-half hour in order to obtain comparative data on the initial rate of exosmosis under different conditions.

We have, of course, no indication from the above regarding the exosmosis or conductivity curve during the 5-day interval. Subsequent work shows that it is very probable that the curve rose considerably in the case of the untreated and the oven-treated cultures and then fell, at the end of 5 days, to a position lower than that of the initial reading, due to the absorption being greater than the excretion after the first 2 or 3 days.

Let us turn now to the results obtained with the fresh seedlings grown in the same water in which the first crop had remained for 5 days under the conditions indicated above. After the second crop had been growing in this medium for just 15 days the green weight of tops of the 4 cultures in each group

was determined, with the following results, the figures representing the average green weight of tops in each culture:

Previous crop untreated	3.39 grams
Previous crop treated with illuminating gas	4.38 grams
Previous crop in oven 1 and 2 hrs. at 60–33°C	3.35 grams

V. Effects of Anesthetic Vapors

For this work the method used was to place the cultures (in some cases the medium also, in which instances the roots were in the water during exposure, and in other cases only the plants themselves, thus exposing the roots directly to the vapor) under bell jars into which the anesthetics were subsequently placed. In the case of ether and chloroform a meas-

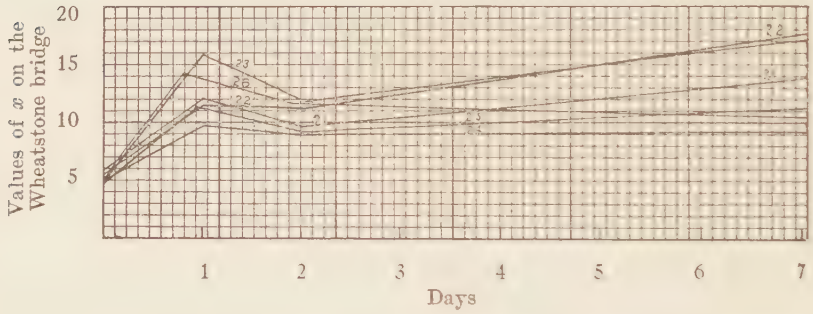

Fig. 1. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment with anesthetics, as follows: No. 21, ether vapor, 1 minute, roots exposed; No. 22, control—roots exposed under bell jar 1 minute; No. 23, ether vapor, 2 minutes, roots exposed; No. 24, ether vapor, 5 minutes, roots exposed; No. 25, ether vapor, 10 minutes, roots exposed; No. 26, ether vapor, 15 minutes, roots exposed. The plants used were 39 days old. The first reading in each case is of the distilled water before the roots were placed in it.

ured amount of these agents was placed in an open evaporating dish under the bell jar, and after the treatment the residue was measured to determine the amount which had evaporated; in the case of the illuminating gas, however, the agent was run in until the air in the bell jar was more or less completely replaced. Where the plants alone were placed under the bell jars they were carefully attached by cheese-cloth bands to the leg of an inverted tripod, over which the bell jar was then placed.

In figures 1 and 2 are shown the results of treatment with ether and illuminating gas for varying periods of time. The plants for this experiment were 39 days old at time of treatment and had roots in good condition and well developed. The

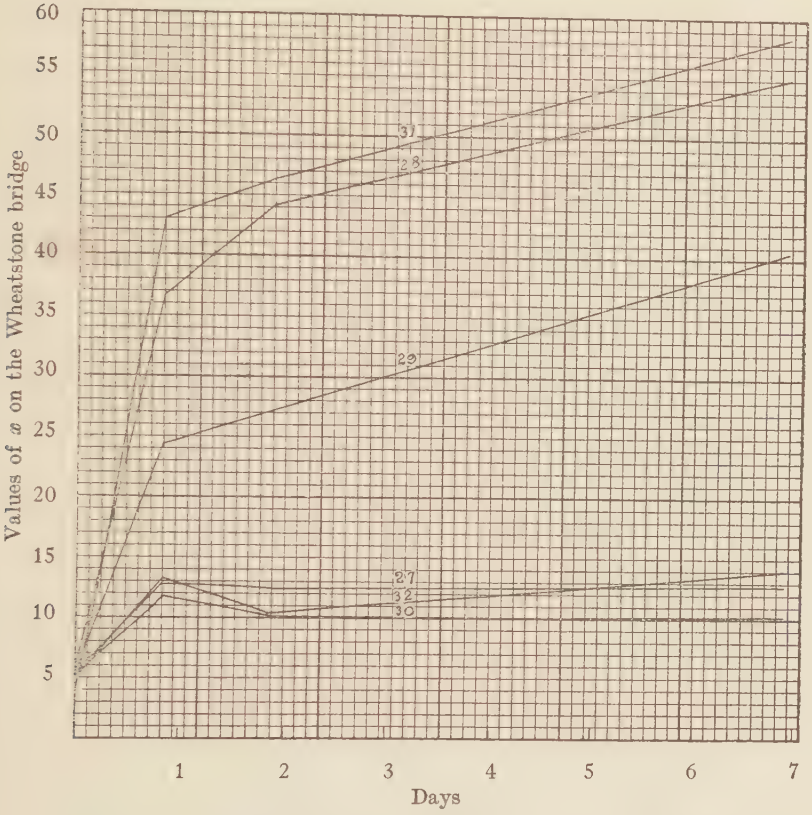

Fig. 2. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment with anesthetics, as follows: No. 27, illuminating gas, 5 minutes, roots exposed; No. 28, illuminating gas, 10 minutes, roots exposed; No. 29, illuminating gas, 15 minutes, roots exposed; No. 30, control—roots exposed under bell jar 15 minutes; No. 31, ether vapor, 3 hours, roots in tumbler; No. 32, control—under bell jar 3 hours, roots in tumbler. The plants used were 39 days old. The first reading in each case is of the distilled water before the roots were placed in it.

first conductivity readings of the water were taken before the plant roots were introduced. As seen from the plotted results the ether had no effect on the exosmosis when the duration of the exposure ranged from 1 to 15 minutes; after 3

hours exposure, however, the exosmosis was pronounced, even when the roots were not in direct contact with the vapor.

An exposure of only 5 minutes to illuminating gas produced no effect, but one of 10 or 15 minutes' duration caused considerable exosmosis. That the 15-minute exposure should result in less exosmosis than the 10-minute one is an interesting point which finds an analogy, we shall see, at different places throughout the work, where in isolated cases a briefer exposure or milder treatment results in greater conductivity of the medium than a somewhat more prolonged exposure or more severe treatment. Where such a condition exists it is usually found near the boundary line of noticeable effect, and not where the effect is either nil or very pronounced. At this critical point the individual hardihood of the plants themselves seems the most plausible explanation of the difference. As the manipulation methods were exactly similar for any given series it is altogether unlikely that difference in technique was responsible for the variation.

The only plants to sustain any injury were those of cultures 28, 29, and 31. The tops of those in No. 31 drooped immediately after the treatment and soon died, though the leaves remained green; the roots, however, remained entirely normal to all appearances and retained their turgor. This is an interesting point and was referred to above. After 7 days Nos. 28 and 29 plainly showed some injury, but it was slight, and its visible effects were slow in making their appearance. At that time the tops of these cultures showed greater yellowing and drying than did those in the controls, No. 29 being somewhat more affected than No. 28; the roots of both, however, remained normal in appearance.

The greatest contrast between the treated plants and the controls is seen in fig. 3. The effect on the treated cultures corresponds to the duration of treatment, the curves especially showing the difference in the speed of initial exosmosis. It will be seen that the conductivity curves of the controls rise rather high during the first day. This is no doubt due to the effect of rather prolonged exposure of the roots to the air in the bell jar, even though it was saturated with water vapor.

Fig. 3. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment, as follows: No. 33, illuminating gas, 15 minutes, roots exposed; No. 34, control—roots exposed under bell jar 15 minutes; No. 35, illuminating gas, 30 minutes, roots exposed; No. 36, control—roots exposed under bell jar 30 minutes; No. 37, illuminating gas, 1 hour, roots exposed; No. 38, control—roots exposed under bell jar 1 hour. The plants were 22 days old when treated. The first readings were taken in the various cultures after the roots had been in distilled water for the following periods: No. 33, 35 minutes; No. 34, 1 hour and 15 minutes; No. 35, 1 hour and 13 minutes; No. 36, 1 hour and 25 minutes; No. 37, 1 hour and 5 minutes; No. 38, 1 hour and 16 minutes.

The subsequent decline in the curve, however, is characteristic for normal root tissues. It is also seen here that the 15-minute exposure to illuminating gas resulted in a greater rise in the conductivity curve than did a similar exposure in the case of the cultures recorded in fig. 2. That this is due to the different ages of the plants in the two cultures was borne out by treatment of plants of different ages with other agents. The older the tissues the more resistant they become to the toxic substance. McCool ('13) was the first to point this out, in his experiments with manganese chloride, and we see that it here holds for anesthetics as well.

Figure 4 shows the effect of illuminating gas at different intervals when only the tops are exposed directly to the gas, the roots meanwhile remaining in distilled water. The plants were affected in proportion to the duration of treatment. The tops of No. 39 were only very slightly injured, so that there was practically no difference between them and the tops of the controls; No. 41 was affected more; and No. 43 still more, finally dying, after progressive drooping and yellowing. But here again the roots of the treated plants were in all respects similar to those of the controls and entirely unaffected, visibly, even though exosmosis was considerable. In such cases it was also presumed that the excreted substances came in part from the tops and that here we had an illustration of the downward flow of food materials which occurs in plants under natural conditions. This presumption was considered experimentally as follows:

Some cultures were placed under a bell jar and treated with illuminating gas as before, the roots meanwhile being in distilled water. The tops of one culture were not cut off, while those of another were removed just before treatment, and finally those of a third were removed just after treatment. The controls were not treated, but their tops were cut off immediately after the roots were placed in the distilled water. The treated plants all gave approximately the same exosmosis, which was considerably more than that from the controls. A point to be noted here is that even though the treated tops which were not cut off were very much affected,

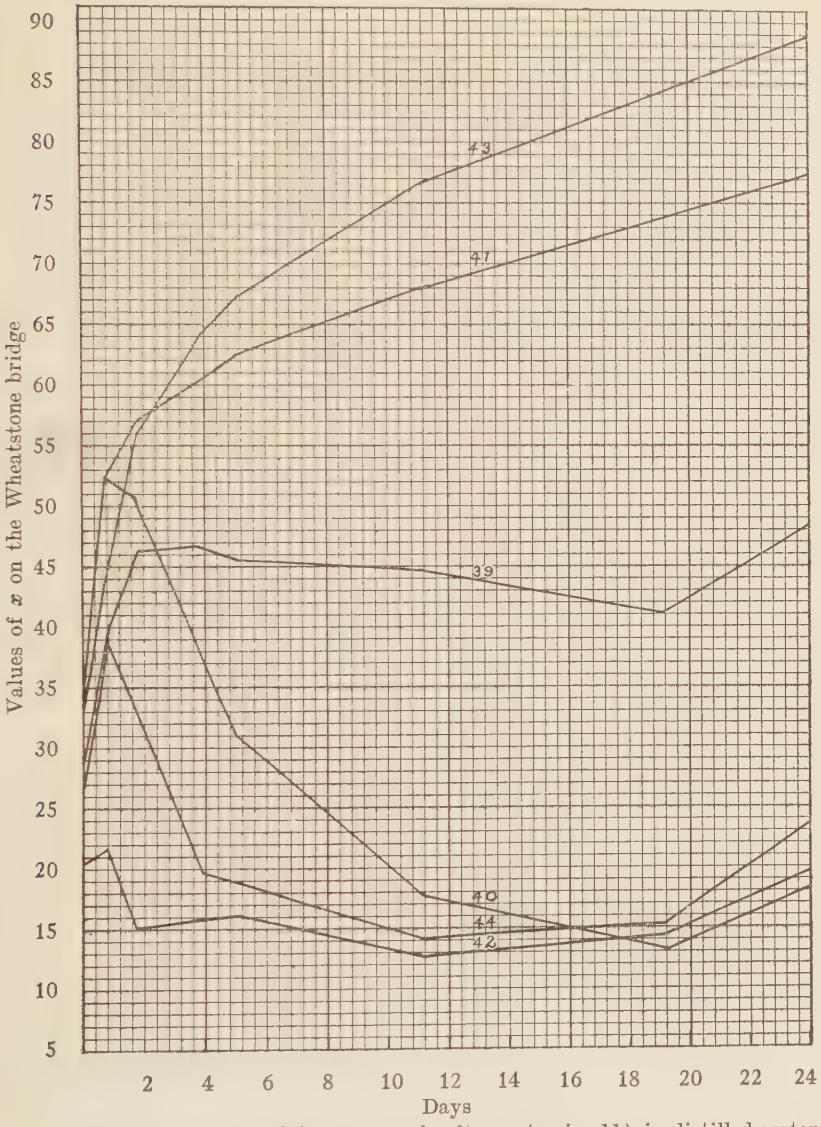

Fig. 4. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment, as follows: No. 39, illuminating gas, 15 minutes, roots in tumbler; No. 40, control—under bell jar 15 minutes, roots in tumbler; No. 41, illuminating gas, 30 minutes, roots in tumbler; No. 42, control—under bell jar 30 minutes, roots in tumbler; No. 43, illuminating gas, 1 hour, roots in tumbler; No. 44, control—under bell jar 1 hour, roots in tumbler. The plants were 22 days old when treated. The first reading was taken in the various cultures after the roots had been in the distilled water subsequent to the treatment for the following periods (but to these periods should be added the time the cultures were under the bell jar, for the roots were in the distilled water during that interval also): No. 39, 2 hours and 12 minutes; No. 40, 2 hours and 23 minutes; No. 41, 2 hours and 20 minutes; No. 42, 2 hours and 30 minutes; No. 43, 2 hours and 11 minutes; No. 44, 2 hours and 23 minutes.

the roots meanwhile remaining practically normal, transpiration no doubt still continued. It remains an open question, however, whether such transpiration caused lower conductivity readings, due to the consequent absorption of electrolytes, than would have been the case had there been no, or only slight, transpiration, as in the cases where the tops were removed. The roots in all the cultures remained turgid and practically

TABLE II

EFFECTS OF ILLUMINATING GAS ON THE EXOSMOSIS FROM THE ROOTS OF PLANTS UNDER VARIOUS CONDITIONS

Culture no.	Treatment	Interval in dist. H_2O before first reading	Conductivity Readings*			
			After first interval	After 24 hrs.	After 88 hrs.	Increase‡ over dist. H_2O after 88 hrs.
1 and 2	Controls in dist. H_2O, no gas treatment. Tops cut off immediately after placing roots in dist. H_2O....	10 hrs.	33.6	37.9	41.8†	35.8†
3	Illuminating gas 1 hr.; roots in tumbler. Tops not cut off.....	1 hr., 17 min.	18.4	38.9	56.7	50.7
4	Illuminating gas 1 hr.; roots in tumbler. Tops cut off immediately after exposure.....	1 hr., 23 min	23.4	46.6	61.6	55.6
5	Illuminating gas 1 hr.; roots in tumbler. Tops cut off just before exposure.....	1 hr., 28 min.	18.6	41.2	60.5	54.5

* Readings represent the values of x on the Wheatstone bridge, resistance in box being 9,110 ohms.

† After 99 hours.

‡ The average reading of the distilled water before roots were placed in it was approximately 6.0.

normal. The higher readings of the treated cultures whose tops had been removed, over those of the untreated controls are to be considered as due to the effect of the illuminating gas, even though in one case only part of the plants was exposed to the agent. The results of this experiment are given in table II.

The results of ether vapor treatment for different periods are seen in fig. 5. An interesting point in this connection is the decline in the curves of the treated plants comparable in

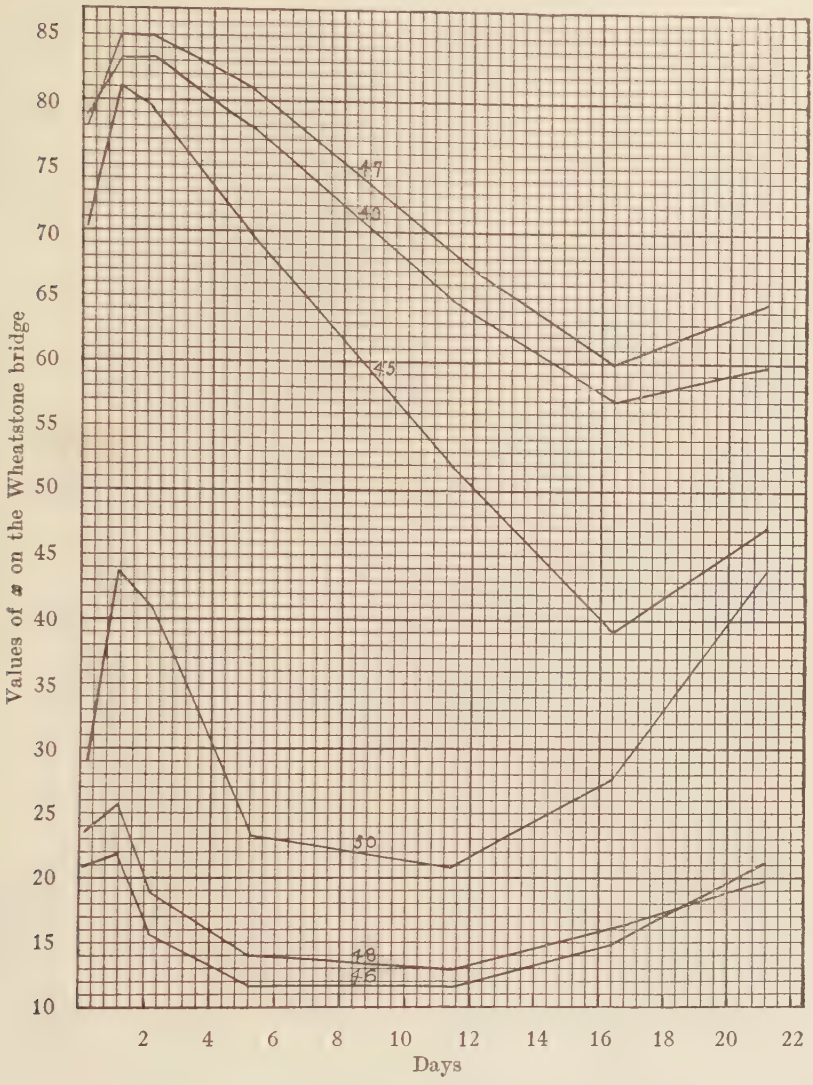

Fig. 5. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment, as follows: No. 45, ether vapor, 30 minutes, roots exposed; No. 46, control—roots exposed under bell jar 30 minutes; No. 47, ether vapor, 1 hour, roots exposed; No. 48, control—roots exposed under bell jar 1 hour; No. 49, ether vapor, 2 hours, roots exposed; No. 50, control—roots exposed under bell jar 2 hours. The plants were 25 days old at the time of treatment. In culture 49, 17cc. of the initial 50cc. of ether remained at the end of the 2 hours. The first reading plotted in each case was taken after the roots had been in the distilled water subsequent to the treatment for the following periods: No. 45, 1 hour and 1 minute; No. 46, 1 hour and 12 minutes; No. 47, 1 hour and 27 minutes; No. 48, 1 hour and 38 minutes; No. 49, 1 hour and 17 minutes; No. 50, 1 hour and 28 minutes.

some respects to that in the curves obtained from normal plants. A distinction should be made here, however, from the causal agency in this decline in conductivity and the anesthetic reversibility that Osterhout ('13) describes. The decline in the curve indicates that the absorption of electrolytes by roots occurs at a greater rate than they are excreted, for both processes, absorption and excretion, are undoubtedly going on and the curve represents the proportionate amounts of each for any given time. Thus if A represents the excretion and B represents the absorption, the curve declines when B is greater than A, and inclines when A is greater than B. Hence the curve may be represented as $A - B = C$, where C represents the number of ions or charge-carriers in the solution. The tops of the treated plants showed no visible effects whatever when compared with the controls. The roots of No. 45 were very slightly affected, but those of Nos. 47 and 49 were considerably so and to about an equal degree, as shown by flaccidity, root coloration, and the colored and turbid appearance of the medium; the tops, however, continued normal for 21 days after the treatment. Hence the metabolic processes no doubt proceeded unimpaired in many respects, as did also transpiration. The decline of the conductivity curve therefore represents merely a partial return to normal conditions. But the higher conductivity of the medium shows greater exosmosis than from the normal plants. This is due to the unalterable and invariable (and not reversible) effect of the anesthetic upon certain cells. Culture 50 shows in the higher position of its curve, as compared with the other controls, an effect that is no doubt due to the 2-hour exposure of the roots to the air in the bell jar.

As seen in fig. 6 no marked results followed the ether application for one-half to two hours when the roots were in the water during the treatment, though a slight rise is evident for the culture exposed 2 hours. No visible effects were produced on either the tops or roots.

Comparing the effects on plants of an ether vapor-saturated atmosphere with those produced by an illuminating gas-saturated atmosphere, it is thus seen that illuminating gas is much

more injurious than is ether vapor under the conditions of the experiment. Equal amounts of each might give different results, however. The gas used was a mixture of water- and coal-gas with a specific gravity of .62 as compared with air;

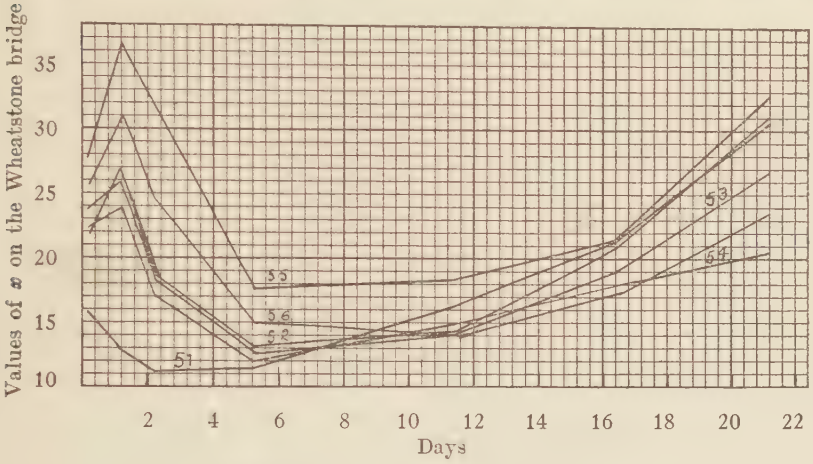

Fig. 6. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment, as follows: No. 51, ether vapor, 30 minutes, roots in tumbler; No. 52, control—under bell jar 30 minutes, roots in tumbler; No. 53, ether vapor, 1 hour, roots in tumbler; No. 54, control—under bell jar 1 hour, roots in tumbler; No. 55, ether vapor, 2 hours, roots in tumbler; No. 56, control—under bell jar 2 hours, roots in tumbler. The plants were 25 days old at the time of treatment. In culture 55, 60cc. of the initial 100cc. of ether remained at the end of the 2 hours. The following periods represent the time elapsing in the various cultures between the removal of the cultures from the bell jar and the taking of the first reading (to which period should be added the duration of treatment, for the roots were in the distilled water during that time also): No. 51, 1 hour and 24 minutes; No. 52, 1 hour and 46 minutes; No. 53, 1 hour and 51 minutes; No. 54, 2 hours and 6 minutes; No. 55, 1 hour and 39 minutes; No. 56, 1 hour and 50 minutes.

one of the daily samples analyzed by the gas company (the officials of which kindly supplied the writer with the data and informed him that they may be considered an approximately fair average) showed the following constituents:

CO_2	3.0%
O_2	.5%
Illuminants (unsaturated hydrocarbons, e. g., ethylene and acetylene)	7.0%
CO	16.1%
CH_4	25.6%
H_2	42.8%
N_2	5.0%

Crocker and Knight ('08), in their work on the question of injury by illuminating gas and its constituents, concluded that "there is much evidence that indicates that the toxic limits of illuminating gas upon these flowers [carnations] is determined by the ethylene it contains." They used a small greenhouse of 1.69 cubic meters' capacity in which they placed potted plants for varying intervals, specified amounts of gas being introduced. The buds were easily injured but the vegetation was apparently not affected even after an exposure of about 72 hours, during which 10 liters of gas had been introduced, 2 or 4 liters at a time. The method was therefore somewhat different from the one employed by the author, in which the plants were placed in an atmosphere saturated with illuminating gas, but for a much shorter period. The underlying cause of the effect in both cases, however, is probably the same.

The etherization of plants as a practical process has been in operation for many decades, especially as a means of hastening the activities of plants, particularly of bringing them into bloom earlier. Some experimental work has also been done, as we have seen, on the effect of such treatment (though in most cases only when the anesthetics were in solution) upon the exosmosis of non-electrolytes, as determined by various methods, from plant or animal cells. It is interesting, therefore, to observe the exosmotic phenomena of electrolytes when the plants are anesthetized under various conditions.

To determine whether the amount of substance excreted corresponded to the conductivity readings, the water in the tumblers was evaporated and the residue weighed. The following are the results:

Total wt. of substance from illuminating gas-treated cultures (Nos. 33, 35, 37, 39, 41, and 43).....0.1514 grams
Total wt. of substance from ether-treated cultures (Nos. 45, 47, 49, 51, 53, and 55).....0.0674 grams
Total wt. of substance from the 12 controls.....0.1077 grams
Total wt. of substance from 6 controls, therefore.....0.0538 grams

We may obtain a rough basis for estimating this residue in terms of NaCl by comparing the figures just given with the data on a previous page which gave the corresponding specific conductivity values for some values of x on the Wheat-

stone bridge and also for various concentrations of NaCl. Thus 0.15 gram residue was obtained from 1500 cc. of the media from illuminating gas-treated cultures. This is equivalent to 0.10 gram in 1 liter, which in terms of NaCl would be

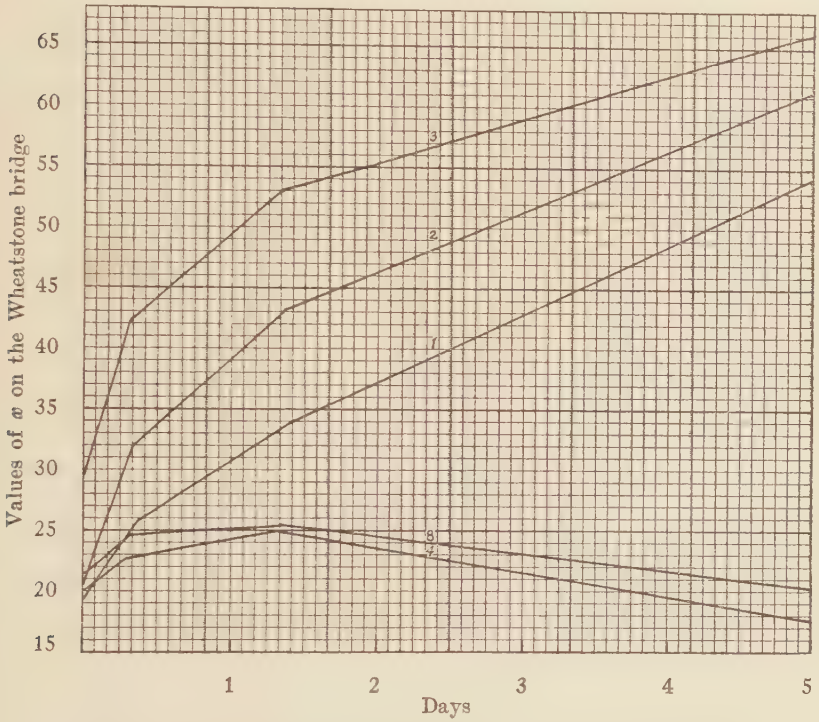

Fig. 7. Conductivity curves of cultures (series 14) in distilled water subsequent to treatment, as follows: No. 1, temperature of —6 to —2°C., 1 hour, roots in tumbler; No. 2, temperature of —6 to —2°C., 2 hours, roots in tumbler; No. 3, temperature of —6 to —2°C., 3 hours, roots in tumbler; No. 4, control—room temperature, roots in tumbler; No. 8, control—room temperature, roots in tumbler. The plants were 23 days old at the time of treatment. The first reading was taken in each case after the roots had been in the distilled water for the following respective periods (cultures 1–3 were being treated during part of that time): No. 1, 1 hour and 47 minutes; No. 2, 2 hours and 29 minutes; No. 3, 3 hours and 45 minutes; No. 4, 3 hours and 6 minutes; No. 8, 2 hours and 2 minutes.

approximately N/500. The specific conductivity of N/500 NaCl is about 25×10^{-5}, the x value of which on the Wheatstone bridge is 85. The average final reading of the 6 cultures treated with illuminating gas is 79.5. Hence the residue in terms of NaCl would be in the neighborhood of N/500.

VI. Effects of High and Low Temperatures

After the preliminary experiments noted above on the effect of heat had been carried out it was desired to study the question further and determine the resulting exosmosis curves at the extreme temperatures, high and low. The preliminary experiments had involved temperatures requiring a considerable time interval to produce positive results. The data now to be presented concern temperatures sufficient in themselves to effect decided injury in a very short period. By varying the time factor, therefore, results could readily be obtained on both sides of the point of injury.

For the experiment, the results of which are plotted in fig. 7, cultures were set out of doors for the time indicated, directly exposed to the winter temperature. The tops showed some signs of freezing after a few moments, but the effects did not become noticeably worse until the cultures were brought inside, when all the plants in each culture immediately drooped over the wire supports and became entirely limp, and soon died. The tops did not yellow, but retained the green color after death. Except for the root tips of the plants in No. 3, which were slightly brown at the end of 5 days, all the roots of the treated plants remained turgid, white, normal, and in healthy condition. This is interesting in view of the fact that while no ice was formed in No. 1, there was a slight fringe of it between the water and the tumbler in No. 2, and a hollow cylinder of ice one-fourth inch thick formed next to the tumbler wall in No. 3. In the last-mentioned culture there was also a film of ice over the surface of the water and the roots were frozen to the ice mass so that on lifting the plants from the tumbler the mass of ice adhered to the roots. The first readings were taken only after the ice had melted. The temperature at first was —6° C. but by the end of the first hour it had risen to —2° C., where it remained practically constant for the balance of the interval.

At a temperature of —6.5° C. it is seen by reference to fig. 8 that while for exposures of the plants alone (the roots being out of the water) of 2 and 3 minutes, marked exosmosis imme-

diately results, exposures of 1 minute or ½ minute produce no results. Culture 13 has rather high exosmosis for a control,

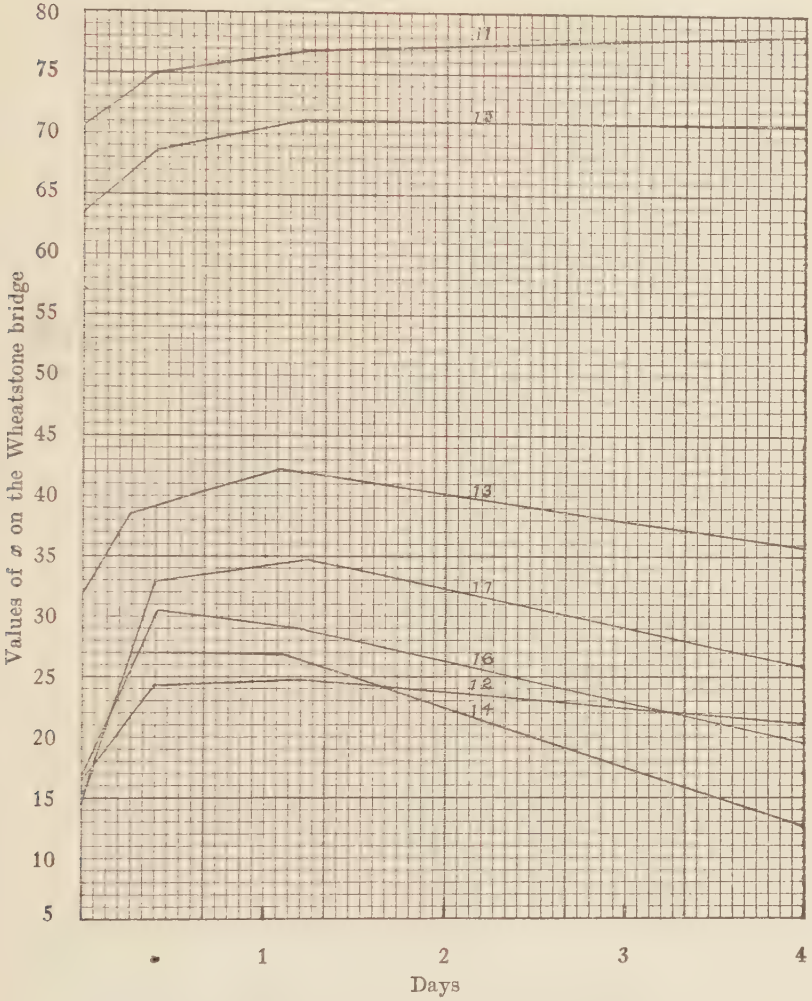

Fig. 8. Conductivity curves of cultures (series 14) in distilled water subsequent to treatment, as follows: No. 11, temperature of — 6.5°C., 3 minutes, roots exposed; No. 12, control—roots exposed to laboratory temperature 15 minutes; No. 13, control—roots exposed to laboratory temperature 30 minutes; No. 14, control—roots exposed to laboratory temperature 5 minutes; No. 15, temperature of — 6.5°C., 2 minutes, roots exposed; No. 16, temperature of —6.5°C., 1 minute, roots exposed; No. 17, temperature of —6.5°C., one-half minute, roots exposed. The plants were 24 days old at the time of treatment. The first reading was taken in each case after the roots had been in the distilled water for exactly 30 minutes subsequent to the treatment.

but this is readily accounted for by the exposure of its roots to the atmosphere of the laboratory for 30 minutes, a condition noted in other cases above.

Cultures 9 and 10 of this series, the results from which are not represented because both tops and roots were killed outright, the resulting exosmosis therefore being immediate and high (x being about 88.0 cm.), were exposed for 15 and 33 minutes respectively to a temperature of — 6.5° C., the roots being out of the medium. In a very short time, on returning them to the laboratory, the tops wilted and drooped over the supporting wires and the roots became very flaccid. In the case of No. 11, however, an interesting gradation or intermediate condition was observed between it and Nos. 9 and 10 on one hand and between it and the controls on the other. While the tops in No. 11 wilted and drooped somewhat soon after being returned to the higher temperature of the laboratory, they did not become entirely limp and the roots were only slightly less turgid than those of the controls. Even after 4 days the tops of No. 11 were not drooping much, though the tips of the branches and the upper leaves were dead; the lower part of the stems and the lower leaves remained green and normal. The lateral roots and the older part of the main roots remained nearly normal, but the tips of the latter were flaccid and shrunken for about 2 inches. Culture 15 showed a very slight flaccidity in the tops and roots soon after the treatment, and after 4 days some of the younger leaves and the tips of the older leaves were blackened, curled, and dried somewhat, but the great part of the tops remained normal in appearance; the roots were slightly flaccid at the tips, but were in general practically normal. Cultures 12, 13, 14, 16 and 17 were normal in respect to both roots and tops.

The interval between 15 and 30 minutes is shown in fig. 9 to be the critical period for the pea plants exposed in a tumbler to a temperature of from — 2° C. to — 2.5° C., for an exposure of 30 minutes caused considerable exosmosis, while one of 15 minutes gave a curve approximately that for normal plants.

To contrast the effects of low and high temperatures, Nos.

25–28 inclusive are plotted in the same figure with Nos. 18 and 23. With plants enveloped in a steam bath the injury, as expected, is very speedy and effective. Even one-half minute —when the roots are exposed—causes immediate and marked

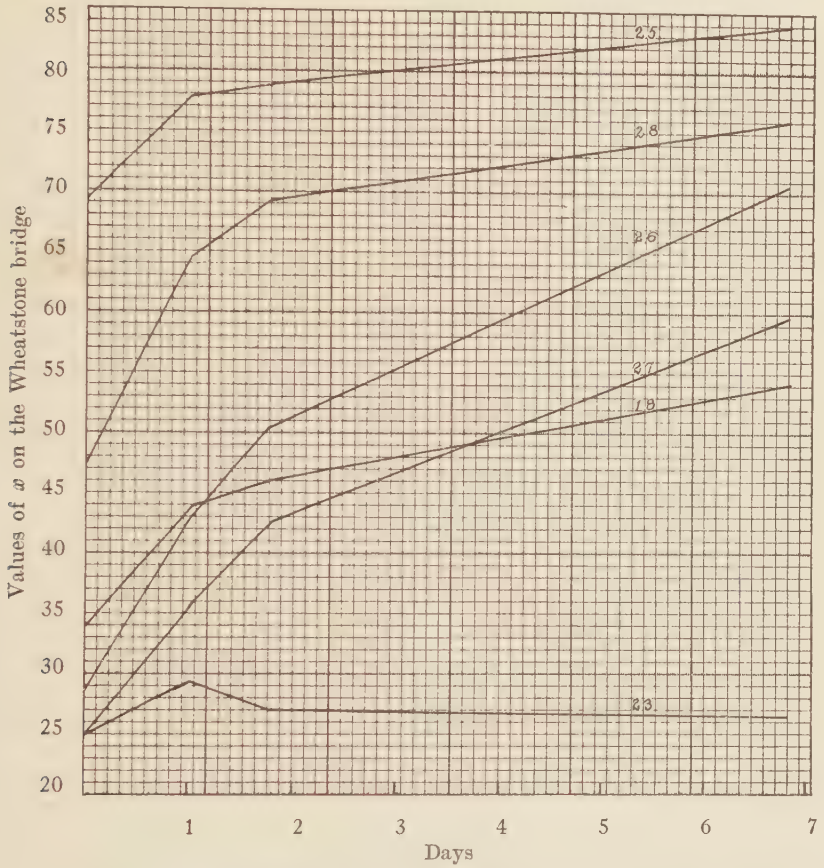

Fig. 9. Conductivity curves of cultures (series 14) in distilled water subsequent to treatment, as follows: No. 18, temperature of —2.5°C., 30 minutes, roots in tumbler; No. 23, temperature of —2.0°C., 15 minutes, roots in tumbler; No. 25, steam, one-half minute, roots exposed; No. 26, steam, 2 minutes, roots in tumbler; No. 27, steam, 1 minute, roots in tumbler; No. 28, steam, 10 minutes, roots in tumbler. The plants were 29 days old at the time of treatment. The first reading was taken in each case after the roots had been in the distilled water for the following respective periods (the cultures whose roots were in tumblers during the treatment were likewise in the distilled water): No. 18, 4 hours and 49 minutes; No. 23, 4 hours and 41 minutes; No. 25, 4 hours and 43 minutes; No. 26, 4 hours and 29 minutes; No. 27, 4 hours and 31 minutes; No. 28, 4 hours and 12 minutes.

exosmosis which is greater than that caused by a 10-minute exposure when the roots are in distilled water meanwhile. The condition of the plants immediately after the treatment and again after 7 days is given in table III. Here again is illus-

TABLE III

CONDITION OF PLANTS AFTER EXPOSURE TO VARIOUS TEMPERATURES

Culture no.	Condition of tops	Condition of roots
	Condition of plants immediately after the treatment:	
18	Considerably flaccid and drooping	Entirely normal
23	Very slightly drooping, nearly normal	Entirely normal
25	Drooping considerably	Normal
26 and 27	Drooping, green and damp	Normal
28	Drooping, green and damp	Apparently practically normal
	Condition of plants 7 days after the treatment:	
18	About half dead and half alive; 3 live stems with green, normal leaves	Entirely normal
23	Almost normal; tips of a few stems killed and some slightly injured, but some stems normal throughout; a few blackened leaves, but for the most part stems and leaves green and normal	Entirely normal
25	Dead	Only very slightly flaccid and nearly normal in appearance
26 and 27	Dead	Practically normal in appearance
28	Dead	Almost normal

trated, therefore, the case where there is considerable exosmosis without very marked visible effects resulting to the root tissues.

The effects of moist heat, as graphically represented in fig. 9, having been considered, we may now turn our attention to fig. 10, where the results are plotted of an exposure of plants to dry heat for short intervals, both with the roots directly exposed and with the roots remaining in the tumbler of water during the treatment.

It is seen that definite and positive exosmosis is obtained after a 4-minute exposure of the unprotected roots. The decline of the curve of No. 29, roots exposed for 2 minutes, is probably best accounted for by assuming greater hardihood of the plants in that culture, or that some condition effected an increase in transpiration. A 1-minute exposure (No. 30) pro-

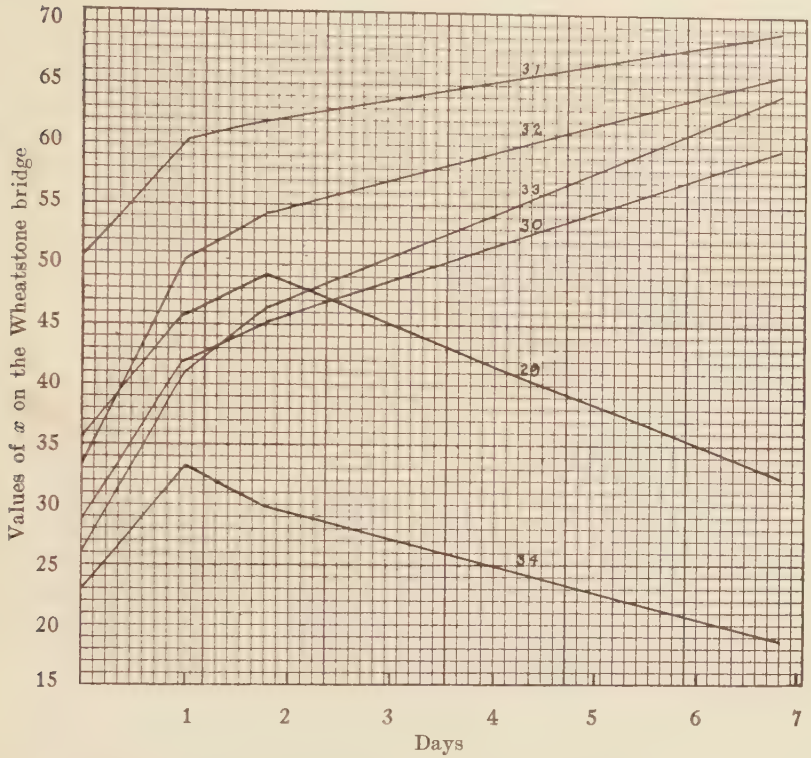

Fig. 10. Conductivity curves of cultures (series 14) in distilled water subsequent to treatment, as follows: No. 29, temperature of 92°C., 2 minutes, roots exposed; No. 30, temperature of 92°C., 1 minute, roots exposed; No. 31, temperature of 92°C., 4 minutes, roots exposed; No. 32, temperature of 92°C., 2 minutes, roots in tumbler; No. 33, temperature of 92°C., 4 minutes, roots in tumbler; No. 34, control—roots in tumbler at laboratory temperature. The plants were 29 days old at the time of treatment. The first reading was taken in each case after the roots had been in the distilled water for the following respective periods (the cultures whose roots were in tumblers during the treatment were likewise in the distilled water during that time): No. 29, 2 hours and 47 minutes; No. 30, 2 hours and 48 minutes; No. 31, 2 hours and 44 minutes; No. 32, 2 hours and 45 minutes; No. 33, 2 hours and 46 minutes; No. 34, 2 hours and 46 minutes.

duced less exosmosis at the beginning than was the case in No. 29, but finally caused more. The same irregularity is also noticed in Nos. 32 and 33. These irregularities near the boundary line of endurance have been discussed above. The tops of Nos. 29–33 were killed by the treatment, but the roots of all remained practically normal in appearance except in No.

31, where the tips were slightly shrunken at first but became almost normal in the water after 7 days.

In the temperature experiments we have thus used the extremes of temperature and have reduced the interval of exposure in order to approach the point at which the effect is just evident.

VII. Effects of Anesthetics in Solution

Having seen some of the effects of anesthetic vapors, we may turn our attention next to the results obtained with anesthetics in solution. In the investigations of others pertaining to the effect of anesthetics, already cited, the result has been almost universally noted that small amounts of anesthetics decrease the exosmosis of coloring matters, etc., while toxic amounts increase it. In most cases this exosmosis was explained on the basis of an alteration in the plasma membrane, small amounts of the anesthetics presumably reducing the permeability and large amounts increasing it. But a point worthy of note is that wherever such effects have been determined the substance under observation was either a colored compound or one of complex organic nature.

Thus Czapek ('11) used the myelin-formation of a tannoid substance, anthocyan, as a basis of observation. From the standpoint of a physical phenomenon, i. e., the lowering of surface tension, his experiments beautifully illustrated the principle under consideration. But from the standpoint of exosmosis in the broader sense we must include electrolytes (salts, bases, and acids) as well as tannin compounds in any discussion dealing with agents affecting exosmosis, and while the critical concentrations which he determined are undoubtedly characteristic of the plants and the compounds studied, the results given herewith show that they are not the limiting concentrations which effect the exosmosis of electrolytes from the roots of certain plants. The limiting concentrations which he found are given in table IV.

Czapek believed the permeability of the plasma membrane was altered under the influence of alcohols, ethers, etc., so that abnormal exosmosis occurred. Whatever may be the expla-

TABLE IV

CRITICAL CONCENTRATIONS (THOSE JUST SUFFICIENT TO CAUSE EXOSMOSIS) OF SOME ORGANIC COMPOUNDS AS DETERMINED BY CZAPEK FOR CERTAIN PLANTS

Agent	Plant	Concentration of agent	Surface tension*
Methyl alcohol	*Echeveria*.....	18% aqueous solution (by volume)	.71
Ethyl alcohol	*Echeveria* and *Saxifraga*	10–13% aqueous solution (by volume).....	.65–.70
Ethyl ether	*Echeveria* and *Viola*	$\frac{1}{4}$–$\frac{1}{2}$ saturated aqueous solution acting for 24 hrs...	.61–.71
Chloroform	*Echeveria*..	Saturated aqueous solution†.... ..	.98
Chloral hydrate	.....	3.09% aqueous solution.....	.93
Ethyl acetate	Saxifraga hairs and variegated leaves of *Oplismenus imbecillus*	3% aqueous solution acting for 12 hrs..	.69–.73
Ethyl acetate	Red beets.....	2% aqueous solution acting for 12 hrs..	

* In terms of water as unity. † After 24 hours the cells had lost all tannin.

nation for the phenomena observed, it will be seen by comparing the results in the following experiments with the data just given that the limiting values found for the exosmosis of electrolytes do not at all correspond to the values found by Czapek for the exosmosis of the tannoid substance.

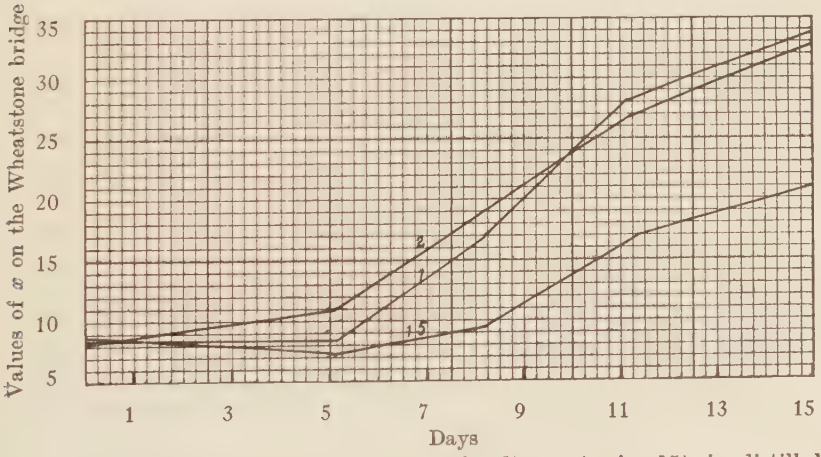

Fig. 11. Conductivity curves of cultures (series 15) in distilled water subsequent to treatment, as follows: No. 1, ether vapor, 15 minutes, roots exposed; No. 2, 1 per cent ether in water, 15 minutes; No. 15, control—roots exposed under bell jar 15 minutes. The plants were 31 days old when treated. In culture 1, 11cc. of the initial 15cc. of ether remained after the 15-minute exposure. In Nos. 1 and 2, the first reading was taken exactly 30 minutes, and in No. 15, 48 minutes, after the roots were placed in the distilled water.

In fig. 11 are shown the results with ether in distilled water and, for comparison, also with ether vapor for the same period. The curves for the ether-treated cultures are closely parallel for the entire period of observation of 15 days, and both are

TABLE V

EFFECTS OF VARIOUS ANESTHETICS ON THE EXOSMOSIS FROM THE ROOTS OF PLANTS

(See curves in fig. 12)

Anesthetic	Time of exposure	Culture no.	Resulting exosmosis
Vapor Treatment, Roots Exposed			
Ether	30 minutes	3	About the same in Nos. 3 and 5
Illuminating gas	15 minutes	5	
Illuminating gas	30 minutes	7	Higher
Chloroform	30 minutes	9	Highest
Treatment with Anesthetics Dissolved in Water			
Ether, 4%	30 minutes	4	High
Ether, 4%	Throughout experiment	11	Highest
Ether, 10%	30 minutes	12	Higher
Illuminating gas-saturated sol'n.	15 minutes	6	Medium low
Illuminating gas-saturated sol'n.	30 minutes	8	Like control
Illuminating gas-saturated sol'n.	Throughout experiment	13	Medium low
Illuminating gas-saturated sol'n. frequently re-saturated	30 minutes	14	Slightly above control
Chloroform, 4%	30 minutes	10	Very high
Controls			
Roots exposed to the air under a bell jar 30 minutes		16	High for control
Roots exposed to the air under a bell jar 30 minutes		17	High for control
Roots not exposed, but in water from first		18	Normal for control

above that of the control. The excretion was nil during the first half hour and at the end of 5 days it was scarcely more, though it may have risen and fallen in the meantime, as no readings were taken in the interim. After 5 days a greater rise in the conductivity curve occurred with the ether-treated cultures than with the control; no apparent effects, however, were produced on either the tops or roots.

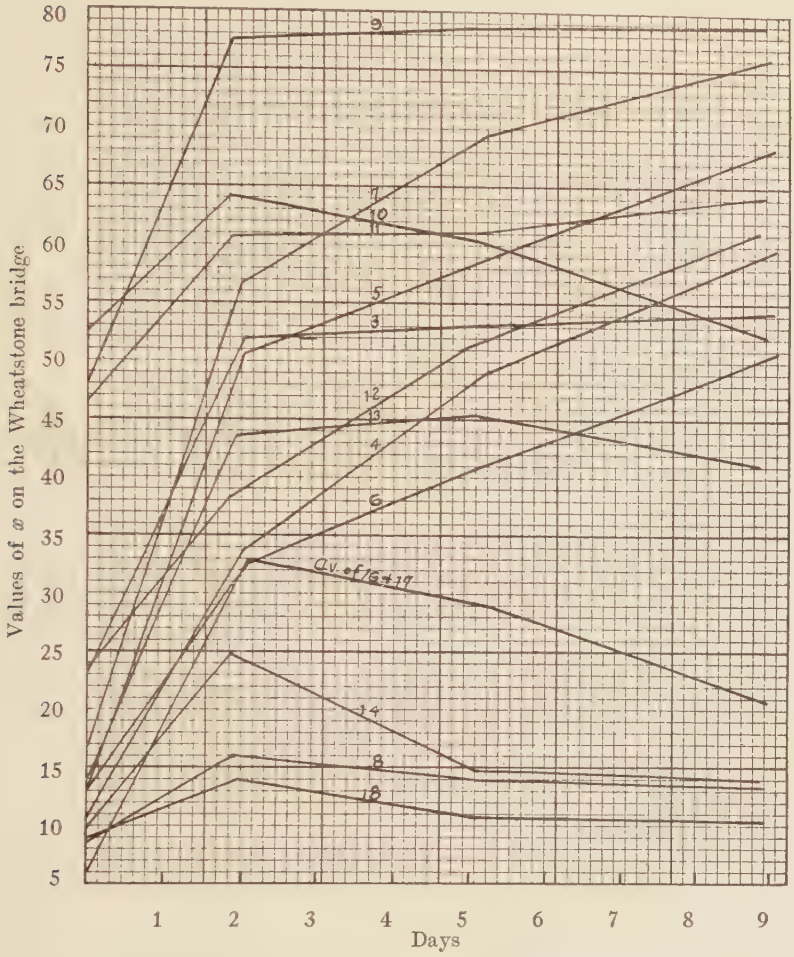

Fig. 12. Conductivity curves of cultures (series 15) in distilled water subsequent to treatment, as follows: No. 3, ether vapor, 30 minutes, roots exposed; No. 4, 4 per cent ether in water, 30 minutes; No. 5, illuminating gas, 15 minutes, roots exposed; No. 6, distilled water saturated with illuminating gas, 15 minutes; No. 7, illuminating gas, 30 minutes, roots exposed; No. 8, distilled water saturated with illuminating gas, 30 minutes; No. 9, chloroform vapor, 30 minutes, roots exposed; No. 10, 4 per cent chloroform in water, 30 minutes; No. 11, 4 per cent ether in water, to the end of the experiment; No. 12, 10 per cent ether in water, 30 minutes; No. 13, distilled water saturated with illuminating gas, to end of experiment; No. 14, distilled water saturated with illuminating gas, 30 minutes (frequently saturated); No. 16, control—roots exposed 30 minutes under bell jar; No. 17, control—roots exposed 30 minutes under bell jar; No. 18, control—roots placed directly into distilled water. The percentages given above refer to volume-per cent. The plants were 37 days old when treated. The first reading was made after the roots had been in distilled water 30 minutes (No. 10, 36 minutes). In all cases treatment preceded placing of the roots in distilled water (conductivity of which was determined except in the cases of Nos. 11, 13, and 18). In culture 3, 17cc. of the initial 25cc. of ether remained at the end of the 30-minute exposure; in culture 9, 23.5cc. of the initial 25cc. of chloroform remained.

To show the comparative effects on the exosmosis from the roots of plants treated with ether, chloroform, and illuminating gas—both when applied as vapor and when introduced into the water—the conductivity curves of fig. 12 were plotted. The results, somewhat classified, are also given in table v. It will be seen that the quantity of anesthetics used and the duration of treatment varied in individual cases.

The indications are, therefore, that for an equal exposure the vapors range in order of effectiveness as follows: ether, least; illuminating gas, more; and chloroform, most. The difference in effectiveness between the ether and the chloroform is especially interesting, more so when we note that 8 cc. of ether were used and only 1½ cc. of chloroform. This would seem to be in harmony with the findings of Graham ('14); he was able to produce liver necrosis by some aliphatic halogen substituted compounds, but not by ether or chloral hydrate.

As regards the fact that No. 11 (4 per cent ether, remaining in the water) has a higher curve than No. 12 (10 per cent ether for 30 minutes) and especially at the beginning, it should be stated that in the case of Nos. 4, 6, 8, 10, 12, and 14, the treatment was given while the roots were in distilled water plus the anesthetics. Following the treatment the roots, after rinsing, were placed in distilled water, and at the end of one-half hour the first reading was taken. In the case of Nos. 11 and 13 the water containing the anesthetic was not replaced by fresh water and the first reading was taken one-half hour after the treatment began. Since the exosmosis during the first half hour is unusually rapid as a result of anesthetic treatment, it will be seen that in replacing the medium at the end of that period, the excreted material was thus discarded for that interval. Hence, such curves represent a secondary exosmosis. The curve of No. 11, therefore, is for total exosmosis, while that of No. 12 is for partial exosmosis.

The condition of the cultures which furnished the results plotted in fig. 11 is given for various periods in table vi.

In fig. 13 the secondary exosmosis after the first half hour is graphically represented for some organic compounds in considerable concentration. The purpose was, of course, to use a

concentration sufficiently effective to give results in a short interval of time. After the treatment the roots were rinsed and placed in distilled water. It is interesting to note that the alcohols used were only slowly effective at first, but that

TABLE VI

CONDITION OF PLANTS AFTER TREATMENT WITH ANESTHETICS

Culture no.	Condition of tops	Condition of roots
Condition of plants 2 days after treatment:		
3 and 4	Slightly subnormal, but almost normal	Somewhat flaccid
5	Practically same as in Nos. 3 and 4 ...	Practically normal
6	Practically same as in Nos. 3 and 4 ...	Practically normal, but somewhat flaccid
7	Practically all dead	Somewhat flaccid
8	Almost normal	Slightly flaccid
9, 10, 11, and 12	Normal	Considerably flaccid
13	Normal	Practically normal
14	Normal	Slightly flaccid
16, 17, and 18	Normal	Normal
Condition of plants 9 days after treatment:		
3	Much dried and yellowed	Practically normal
4	Slightly worse than in No. 3	Considerably flaccid
5	Same as in No. 4	Less flaccid than in No. 4
6	Mostly dried up	Practically normal
7	All dried up	Slightly flaccid
8	Practically normal	Practically normal
9	Practically normal	Considerably flaccid
10	Practically normal	Somewhat flaccid
11 and 12	Almost normal	Somewhat flaccid
13 and 14	Normal	Practically normal
16	Slightly subnormal	Practically normal
17 and 18	Normal	Normal

after 8 days the conductivity readings for those cultures were as high as those of the other cultures. Benzol and toluene produced almost identical effects. The effect produced by chloral hydrate remained constant after 1 day. Ethyl acetate and benzaldehyde were especially effective. The condition of the plants at the end of 8 days is given in table VII.

In fig. 14 are shown the effects of smaller amounts of the same substances, the curves of which are exhibited in fig. 13. Here, however, concentrations only one-fourth as great as those previously employed were used, but the chemicals were allowed to remain in the water during the entire period (or until evaporated, as may have been the case with some).

While the alcohols gave a greater effect than the control, they gave no greater exosmosis than one of the controls in the

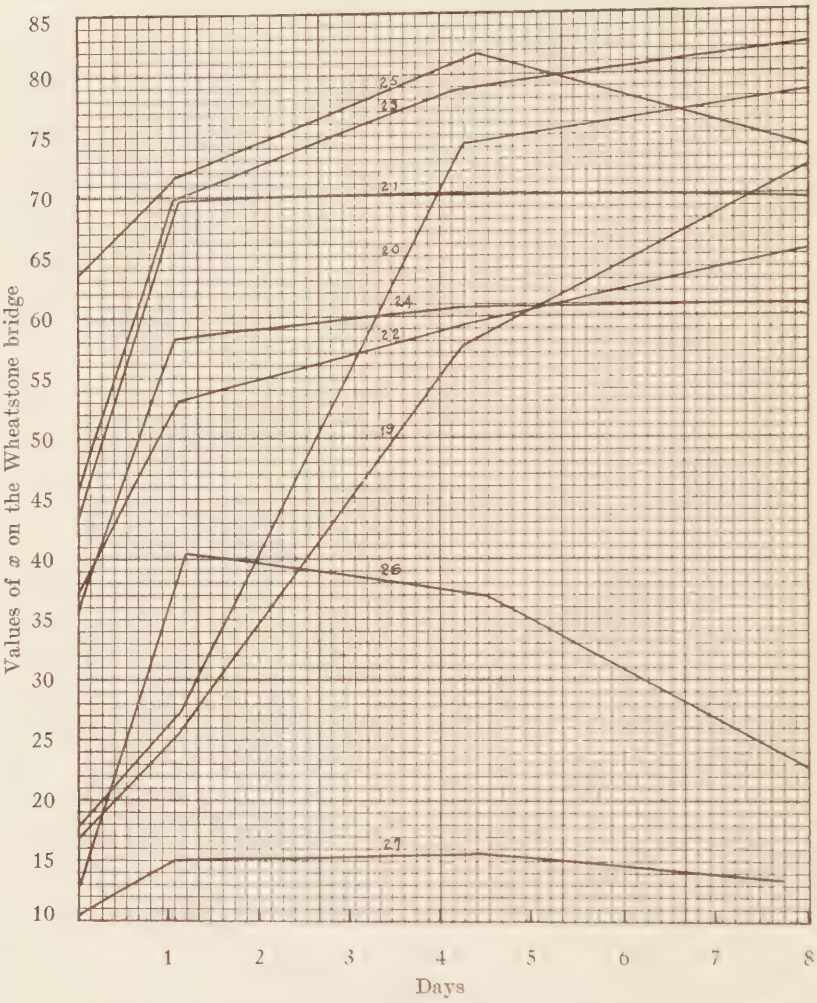

Fig. 13. Conductivity curves of cultures (series 15) in distilled water subsequent to treatment, as follows: No. 19, 4 per cent ethyl alcohol in water, 30 minutes; No. 20, 4 per cent methyl alcohol in water, 30 minutes; No. 21, 4 per cent chloral hydrate in water, 30 minutes; No. 22, 4 per cent benzol in water, 30 minutes; No. 23, 4 per cent ethyl acetate in water, 30 minutes; No. 24, 4 per cent toluene in water, 30 minutes; No. 25, 4 per cent benzaldehyde in water, 30 minutes; No. 26, control—placed directly into distilled water; No. 27, control—placed directly into distilled water. The plants were 38 days old at the time of treatment. The first reading was taken in all cases after the roots had been in distilled water exactly 30 minutes. In the case of the treated plants the roots were in distilled water containing the anesthetic for the specified time, after which they were transferred to the distilled water, the conductivity of which was subsequently determined.

previous figure. The other substances, however, even in the small concentration employed, produced a marked rise in the conductivity of the medium during the first day, after which it remained practically constant. The benzaldehyde and the

TABLE VII

CONDITION OF PLANTS EIGHT DAYS SUBSEQUENT TO TREATMENT WITH EFFECTIVE CONCENTRATIONS OF ANESTHETICS FOR A SHORT PERIOD

Culture no.	Condition of tops	Condition of roots
19	Normal and in good condition	Considerably flaccid
20	Practically normal	More flaccid than those of No. 19
21	Somewhat subnormal	Somewhat flaccid
22	Some stems considerably affected, others almost normal	Considerably flaccid
23	Practically all dead	Very flaccid
24	About the same as in No. 22	Considerably flaccid
25	Considerably subnormal	Very flaccid
26	Normal; many green, vigorous, turgid leaves	Practically normal
27	Practically normal	Normal

ethyl acetate, which themselves give a high conductivity in aqueous solution, should be considered apart from the other substances, which give no such increase. The two substances mentioned are given here merely for the purpose of comparison with the others employed. A 1 per cent solution of ethyl acetate had a conductivity of 65.2 on the Wheatstone bridge, while that of a similar solution of benzaldehyde was 88.6. These corrections should therefore be applied to the curve values in order to obtain the true value of the exosmosis from the roots in those cultures. The condition of the plants after 8 days is given in table 8.

VIII. Effects of Substances Used Singly and Combined in Pairs

It is not the writer's purpose here to go into the historical aspect of the increasingly voluminous work on toxic agents, antagonistic action, and balanced solutions, and the numerous related subjects. But since those subjects have assumed such great importance in the realm of physiology it was thought desirable to consider the effect of certain toxic and unbalanced

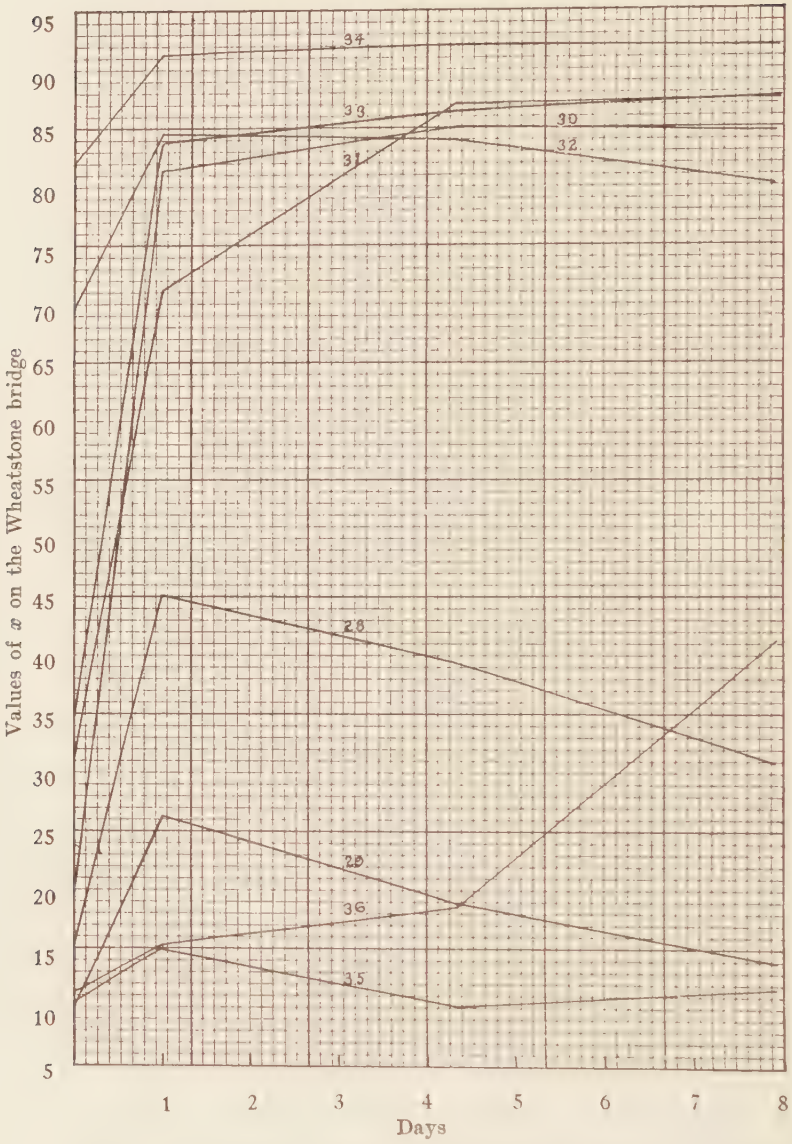

Fig. 14. Conductivity curves of cultures (series 15) in distilled water subsequent to treatment, as follows: No. 28, 1 per cent ethyl alcohol in water, to end of experiment; No. 29, 1 per cent methyl alcohol in water, to end of experiment; No. 30, 1 per cent chloral hydrate in water, to end of experiment; No. 31, 1 per cent benzol in water, to end of experiment; No. 32, 1 per cent ethyl acetate in water, to end of experiment; No. 33, 1 per cent toluene in water, to end of experiment; No. 34, 1 per cent benzaldehyde in water, to end of experiment; No. 35, control—placed directly into distilled water; No. 36, 1 per cent methyl alcohol in water, to end of experiment. The plants were 38 days old at the time of treatment. The first reading was taken in all cases after the roots had been in the distilled water containing the anesthetic exactly 30 minutes. The control, however, was exposed to the distilled water only, the first reading being taken after 30 minutes.

solutions on the exosmosis from plant roots in order to obtain a basis of comparison with the other agents used.

In this connection it might be well to consider more in detail the work of Lillie already referred to in the historical

TABLE VIII

CONDITION OF PLANTS EIGHT DAYS SUBSEQUENT TO TREATMENT WITH LOW CONCENTRATIONS OF ANESTHETICS FOR THE ENTIRE PERIOD

Culture no.	Condition of tops	Condition of roots
28	Somewhat subnormal	Slightly flaccid
29	Almost normal	Considerably flaccid; tips less flaccid than in No. 28 but upper part more so
30	Dead	Considerably flaccid
31	Almost dead	Very flaccid
32–34	Practically dead	Very flaccid
35	Practically normal	Practically normal
36	Practically normal	Somewhat flaccid

review. His work on *Arenicola* and the eggs of *Arbacia* pertains largely to the exosmosis of the pigment and the manner in which they were affected by isotonic salt solutions alone and in the presence of various anesthetics. From the effect observed, he concluded that the salts have a permeability-increasing effect on the plasma membrane which is counteracted by the anesthetics. But in dealing with the question of permeability it would seem that we must take into consideration the effect on the exosmosis, not only of any contained pigment, but of electrolytes as well.

It would have been exceedingly interesting, and would have furnished a means of strengthening or shattering his hypothesis, as the case might be, had Lillie also measured the electrical conductivity of the medium in which the Arenicola larvae and the Arbacia eggs were placed and thus determined whether the electrolytes contained in these organisms behaved as did the pigment. It would seem that the work of Loeb ('03), Peters ('04), and others might be considered as suggesting possibilities for electrolytic determinations along this line with marine organisms. Without such facts at hand any general conclusions in regard to permeability effects based on the coloring matter only must be considered imperfect. What

the reaction may be between the anesthetics and the larval pigment is another question which Lillie does not touch upon. In a recent article Miss Wheldale ('14), in discussing the natural and artificial extracts of plants, states that whereas artificial anthocyanin is soluble in ether the natural anthocyanins are not. May we not have a similar effect in the pigments concerned? Small amounts of the anesthetics may render those pigments insoluble and in that manner prevent their exosmosis rather than by bringing about any considerable alteration of the membrane; larger amounts of the anesthetics would act chemically on the membrane to a point of disintegration sufficient for the physical escape of the pigment.

It will be seen from the following experiments that in the case of roots of *Pisum sativum* certain salts caused a marked exosmosis of electrolytes. In the presence of anesthetics this exosmosis was not decreased or prevented, as Lillie found in the case of the pigments referred to, but was even increased. Hence these results do not indicate any permeability-decreasing action on the part of the anesthetics and are therefore in harmony with the findings of Dixon and Atkins ('13) and others. Another interesting condition is seen in the exosmosis resulting from single and combined salts acting for different periods of time. It was expected that such results would correspond with those obtained on plant-growth studies of antagonistic action between various nutrient and non-nutrient salts. That equally as high, or in some cases higher, exosmosis values were obtained from combined salts as from single salts is an unexpected and interesting result.

As previously indicated, the method used was to place the plants in the various solutions for the period specified and then transfer them, after careful rinsing of the roots, to distilled water in which the conductivity readings were to be taken. It was ascertained that the rinsing was effective in removing electrolytes from the roots. Figures 15 and 16 show the results for the briefer treatments with certain salts, and it is there seen that for a period of treatment less than 17 hours the N/20 $MgCl_2$ has no effect. While in the case of the culture treated for one-half hour with the $MgCl_2$, the conductivity

reading was higher at the end of four days than in the other cultures (2–5) of that group, and continued higher throughout, this fact loses its significance, as far as comparative effects are concerned, when the curve resulting from a 4-hour

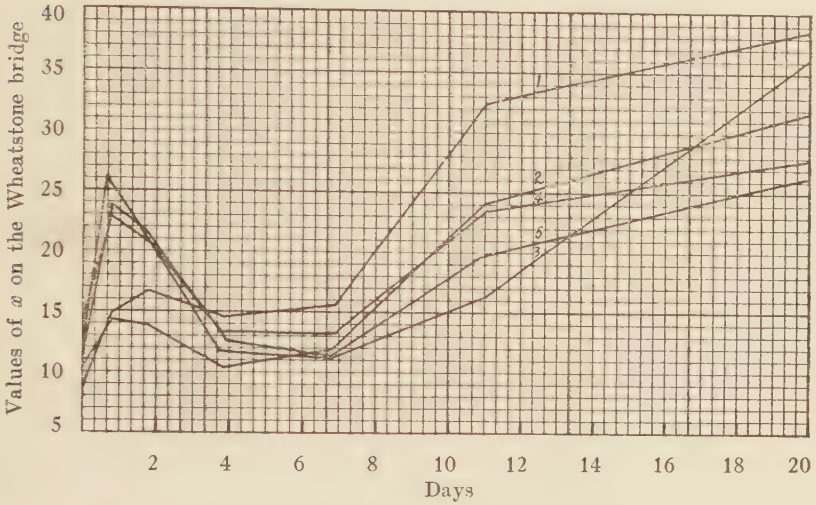

Fig. 15. Conductivity curves of cultures (series 16) in distilled water subsequent to treatment, as follows: No. 1, N/20 $MgCl_2$, 30 minutes; No. 2, N/20 $CaCl_2$, 30 minutes; No. 3, N/20 $MgCl_2$ plus N/20 $CaCl_2$, 30 minutes; No. 4, control—placed directly into distilled water; No. 5, N/20 $MgCl_2$, 4 hours. The cultures were 17 days old at the time of treatment. The first reading was taken in all cases after the roots had been in the distilled water exactly 30 minutes.

treatment with $MgCl_2$ is considered, and should no doubt be interpreted as an individual variation irrespective of treatment. In the case of No. 6, however, the curve for which represents the results of a 17-hour treatment with N/20 $MgCl_2$, we no doubt have a real effect clearly distinguished from the controls.

At the end of 20 days in distilled water following the treatment the tops of Nos. 1–10 were all in about the same condition, those of the treated plants showing no injury. Likewise the roots of Nos. 1–5 and 8–10 were practically normal, with no, or only very slight, flaccidity; those of No. 6, however, were brownish in color and somewhat flaccid, while those of

No. 7 were brownish only in spots, but were of about the same flaccidity as those of No. 6.

Having found that a treatment of 17 hours under the conditions indicated above was not sufficient to yield the most

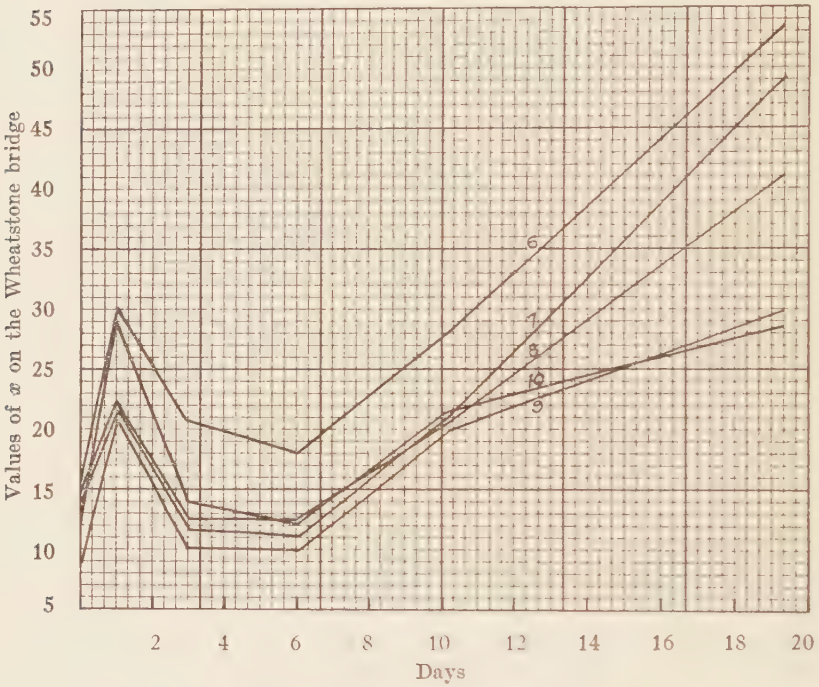

Fig. 16. Conductivity curves of cultures (series 16) in distilled water subsequent to treatment, as follows: No. 6, N/20 $MgCl_2$, 17 hours; No. 7, N/20 $CaCl_2$, 17 hours; No. 8, N/20 $MgCl_2$ plus N/20 $CaCl_2$, 17 hours; No. 9, control—distilled water, renewed after 17 hours; No. 10, control—distilled water, not renewed. The cultures were 17 days old at the time of treatment. The first readings were taken in all cases after the roots had been in the distilled water exactly 30 minutes. No. 10 remained in the full nutrient solution until the treated cultures were transferred (after the 17-hour period) from the respective solutions to distilled water.

positive results, it was decided to try stronger concentrations and longer periods. Figure 17 shows the conductivity curves after a period of treatment extending 75 hours. Some interesting results were obtained. N/10 $MgCl_2$ gave the highest readings, closely followed by N/10 $MgCl_2$ plus N/10 $CaCl_2$; the N/20 $MgCl_2$ plus N/20 $CaCl_2$ curve is very similar to that

obtained from N/10 $MgCl_2$ plus N/20 $CaCl_2$, while the N/10 $MgCl_2$ plus N/100 $CaCl_2$ causes a rise higher than that in the two curves just mentioned after the fifth day. It was unexpected that N/20 $CaCl_2$ should exceed N/20 $MgCl_2$ in its

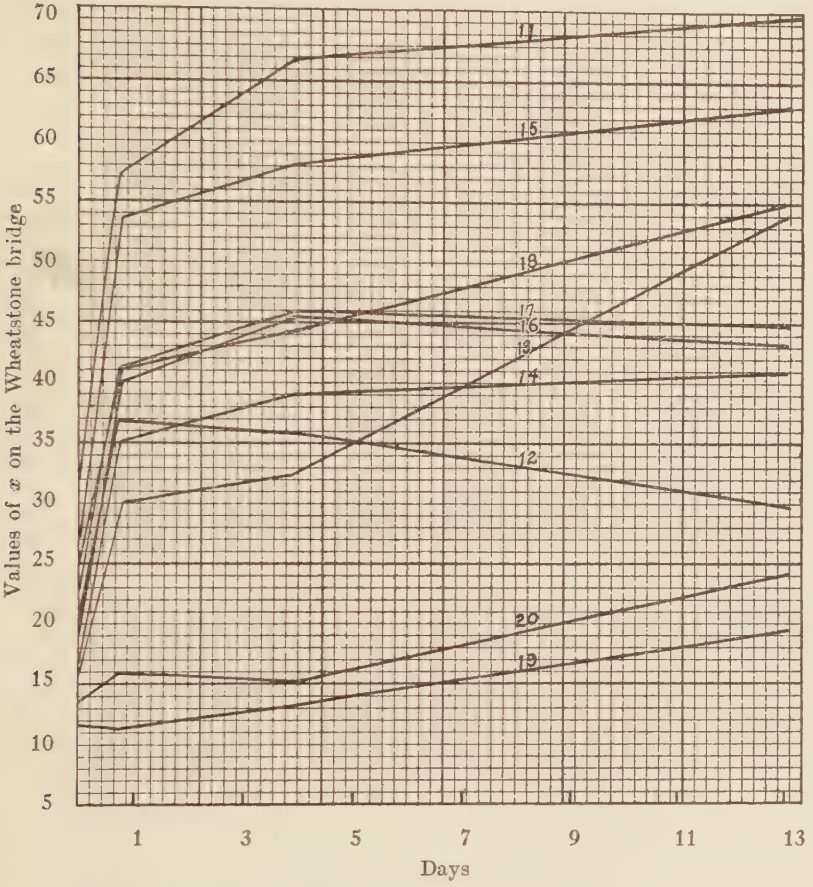

Fig. 17. Conductivity curves of cultures (series 16) in distilled water subsequent to treatment, as follows: No. 11, N/10 $MgCl_2$, 75 hours; No. 12, N/20 $MgCl_2$, 75 hours; No. 13, N/10 $CaCl_2$, 75 hours; No. 14, N/20 $CaCl_2$, 75 hours; No. 15, N/10 $MgCl_2$ plus N/10 $CaCl_2$, 75 hours; No. 16, N/20 $MgCl_2$ plus N/20 $CaCl_2$, 75 hours; No. 17, N/10 $MgCl_2$ plus N/20 $CaCl_2$, 75 hours; No. 18, N/10 $MgCl_2$ plus N/100 $CaCl_2$, 75 hours; No. 19, control—distilled water, renewed after 75 hours; No. 20, control—distilled water, not renewed. The plants were 21 days old when treated. The first reading was taken after the roots had been in the distilled water exactly 30 minutes (in No. 20, 75 hours). Nos. 19 and 20 were placed in distilled water at the same time that the cultures to be treated were placed in their respective solutions.

effect on exosmosis and that the conductivity curve resulting from treatment with N/10 $CaCl_2$ should rise so high at the end.

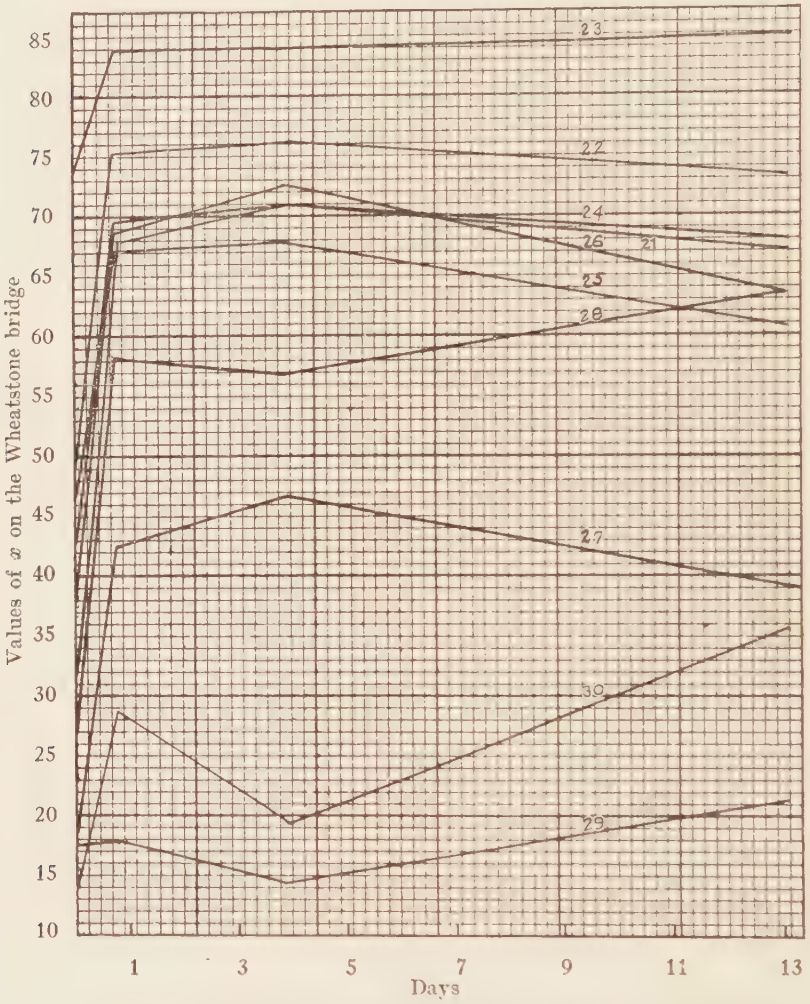

Fig. 18. Conductivity curves of cultures (series 16) in distilled water subsequent to treatment, as follows: No. 21, N/10 NaCl, 75 hours; No. 22, N/10 KCl, 75 hours; No. 23, N/10 NaCl plus N/10 KCl, 75 hours; No. 24, N/20 NaCl plus N/20 KCl, 75 hours; No. 25, N/10 NaCl plus N/10 $CaCl_2$, 75 hours; No. 26, N/10 KCl plus N/10 $CaCl_2$, 75 hours; No. 27, N/20 NaCl, 75 hours; No. 28, N/20 KCl, 75 hours; No. 29, control—distilled water, not renewed; No. 30, control—distilled water, not renewed. The plants were 21 days old when treated. The first reading was taken after the roots had been in the distilled water 30 minutes (in No. 29, 75 hours). Culture 30 was placed in distilled water at the end of the 75-hour period, having been in full nutrient solution up to that time.

At the end of 13 days in distilled water following the treatment, the tops of Nos. 11–20 were of the same appearance throughout, i. e., normal. The roots were also practically normal in the case of Nos. 12–20, except for a brownish color on those of Nos. 12, 13, 15, and 16–18, being especially evident in the case of No. 15. In addition to being brown, however, the roots of No. 11 were considerably flaccid.

Figure 18 shows similar relations for NaCl, KCl, and $CaCl_2$. It is seen that KCl is more effective than NaCl in causing exosmosis. Far from ameliorating the exosmotic condition, the treatment with combined NaCl and KCl likewise yields high conductivity readings of the medium, the N/10 concentration of each combined giving the highest. It can not be argued that this effect is due solely to the osmotic pressures of the solutions of the agents in question, for if that were the case we should expect more comparable results on the basis of the osmotic effects of the various solutions at the concentrations used. There is a reduction in the effect when the NaCl and KCl used singly are reduced to concentrations of N/20.

The condition of the plants 16 days after first applying the treatment, or 13 days after being in distilled water, is shown in table IX, from which it is evident that there was great exosmosis with but little or no visible effect accompanying it.

TABLE IX

CONDITION OF PLANTS TREATED WITH VARIOUS SALTS FOR DIFFERENT PERIODS OF TIME

Culture no.	Condition of tops	Condition of roots
21 and 22	Normal	Slightly brown and very slightly flaccid
23	Dead—badly wilted at end of treatment	Very limp and flaccid and brownish
24–28	Normal	Very slightly brownish but practically normal
29 and 30	Normal	Practically normal

That osmotic effects play practically no part in the phenomenon under consideration is indicated from the results of Loeb ('03) on *Gammarus* and those of True ('14) on Lupinus seedlings. The writer also performed experimental work to

determine this point. Solutions of pure saccharose of varying concentrations were used and the effects produced by the same during a period of 24 hours as compared with pure distilled water, measured by the determined conductivity of the medium both during the 24 hours and after (when the plants which had been in the sugar solutions were also placed in distilled water). These results are given in table x. As there seen, no differences were obtained from the different concentrations.

TABLE X

EXOSMOSIS FROM THE ROOTS OF PLANTS IN SUGAR SOLUTIONS AND DISTILLED WATER *

Time of readings	Culture 1 1.28% saccharose sol'n. 24 hours followed by dist. H_2O	Culture 2 2.56% saccharose sol'n. 24 hours followed by dist. H_2O	Culture 3 5.13% saccharose sol'n. 24 hours followed by dist. H_2O	Culture 4 control dist. H_2O throughout changed every day conductivity readings
Conductivity readings of the sugar solutions:				
Before roots placed in the solution	9.4	10.6	10.9	10.9
After 10 hrs	28.0	19.7	30.8	32.9
After 24 hrs	28.9	18.3	29.0	32.9
Increase over original sol'n. during 24 hrs	19.5	7.7	18.1	22.0
Conductivity readings of the distilled water:				
After ½ hr	9.4	8.6	8.8	8.3
After 23 hrs	10.5	11.5	11.0	10.4
After 48 hrs	10.9	11.3	10.2	10.0
Increase over dist. H_2O the first half hour †	3.4	2.6	2.8	2.3
Increase over dist. H_2O during 48 hours †	4.9	5.3	4.2	4.0

* All readings represent values of x on the Wheatstone bridge, the resistance in the box being 9,110 ohms.

† The average reading of the distilled water before placing roots in it was approximately 6.0.

In table xi are shown the effects produced by salts alone as well as by salts plus anesthetics in weak concentrations. It was desired to use approximately the same concentrations of anesthetics as indicated by the work of Lillie ('12), Osterhout ('13), and others. The conductivity of the water containing the anesthetics was not determined after the 53-hour treatment and hence the resulting exosmosis during that interval was not ascertained. But from other experiments on

the effect of anesthetics in solution we have seen that the exosmosis is rapid and considerable during the first day or so and then remains stationary, i. e., the curve becomes hori-

TABLE XI

EFFECTS OF SALT SOLUTIONS USED SINGLY AND COMBINED WITH ANESTHETICS UPON THE EXOSMOSIS FROM THE ROOTS OF PLANTS (PLANTS 40 DAYS OLD WHEN TREATED)

Culture no.	Treatment	Conductivity*				
		Readings		Increase over dist. H_2O**		
		After ½ hr.	After 42 hrs.	1st ½ hr.	Next 41½ hrs.	Total in 42 hrs.
31	N/10 $MgCl_2$, 53 hrs.....	35.4	57.1	29.4	21.7	51.1
32	N/10 NaCl, 53 hrs.....	34.4	60.9	28.4	26.5	54.9
33	N/10 KCl, 53 hrs.....	40.8	63.0	34.8	22.2	57.0
34	0.7% ether in H_2O, 53 hrs.....	10.0	11.6	4.0	1.6	5.6
35	0.7% $CHCl_3$ in H_2O, 53 hrs.....	12.1	36.3	6.1	24.2	30.3
36	0.7% benzol in H_2O, 53 hrs.....	11.3	17.7	5.3	6.4	11.7
37	N/10 $MgCl_2$ and 0.7% ether, 53 hrs.....	41.2	64.7	35.2	23.5	58.7
38	N/10 $MgCl_2$ and 0.7% $CHCl_3$, 53 hrs.....	45.5	57.2	39.5	11.7	51.2
39	N/10 $MgCl_2$ and 0.7% benzol, 53 hrs.....	44.0	49.4	38.0	5.4	43.4
40	N/10 NaCl and 0.7% ether, 53 hrs.....	37.4	59.5	31.4	22.1	53.5
41	N/10 NaCl and 0.7% $CHCl_3$, 53 hrs.....	47.5	62.4	41.5	14.9	56.4
42	N/10 NaCl and 0.7% benzol, 53 hrs.....	49.5	56.0	43.5	6.5	50.0
43	N/10 KCl and 0.7% ether, 53 hrs.....	50.2	76.0	44.2	25.8	70.0
44	N/10 KCl and 0.7% $CHCl_3$, 53 hrs.....	51.7	65.4	45.7	13.7	59.4
45	N/10 KCl and 0.7% benzol, 53 hrs.....	49.2	55.8	43.2	6.6	49.8
46 and 47	Control (dist. H_2O renewed after 53 hrs.)...	10.9	11.9	4.9	1.0	5.9
48	Control (dist. H_2O not renewed).....	15.2†	15.5‡	9.2§	.3	9.5‖
49 and 50	Control (full nutr. until Nos. 31–45 were placed in dist. H_2O).....	12.5	39.0	6.5	26.5	33.0

* All readings represent values of x on the Wheatstone bridge with a resistance in the box of 9,110 ohms.

** The average reading of the distilled water before placing roots in it was approximately 6.0.

† After 53 hours. ‡ After 95 hours. § In 53 hours. ‖ In 95 hours.

zontal. We can therefore safely infer that such was the case here, except possibly in the ether-treated cultures in which the roots and tops showed no effect whatever from the treat-

ment. We can thus account for the fact that the increase in the conductivity of the medium in Nos. 34, 35, and 36, was so slight and so similar to that given by the controls. It will be seen that the anesthetics did not antagonize the salts so far as exosmosis of electrolytes is concerned. The condition of the plants after the 53-hour treatment is shown in table XII.

TABLE XII

CONDITION OF PLANTS FIFTY-THREE HOURS AFTER TREATMENT WITH SOLUTIONS OF SALTS AND ANESTHETICS

Culture no.	Condition of tops	Condition of roots
31	Normal	Yellowish brown, somewhat flaccid
32	Practically normal	Slightly yellow, practically normal
33	Subnormal, drying considerably	Slightly yellow, practically normal
34	Normal	Practically normal
35	Normal	White, but considerably flaccid
36	Almost normal	Very flaccid
37	Practically normal	Yellowish and considerably flaccid
38	Almost normal	Somewhat flaccid
39	Drying considerably	Very flaccid
40	Normal	Almost normal
41	Drying somewhat	Considerably flaccid
42	Drying considerably	Very flaccid
43	Practically normal	Practically normal
44	Drying considerably	Considerably flaccid
45	Drying badly	Very flaccid
46–50	Normal	Practically normal

The concentration of the anesthetics used in the above experiments was near the boundary which would just produce exosmosis. To eliminate such action entirely when these substances were used alone, therefore, the concentrations used were reduced to a point below that at which they cause exosmosis to any appreciable extent, if at all. The results of that series are given in table XIII, where we see again no indications that there is any decreasing effect by the anesthetics on the exomosis induced by salts. On the contrary, the combined salt and anesthetic cause a greater exosmosis than the salt alone.

As measured by the resulting growth of roots, Hibbard ('13) found an antagonistic action between $CuSO_4$ and chloral hydrate. To determine if such action would also hold true in

the case of exosmosis, an experiment was set up, the results of which are given in table XIV. As there seen, there was no decrease in the exosmosis caused by either substance when the two were combined.

TABLE XIII

EFFECTS OF SALT SOLUTIONS USED SINGLY AND COMBINED WITH ANESTHETICS ON THE EXOSMOSIS FROM THE ROOTS OF TREATED PLANTS*

Culture no.	Treatment	Conductivity				
		Readings		Increase over dist. H_2O‖		
		After ½ hr.	After 41 hrs.	1st ½ hr.	Next 40½ hrs.	Total in 41 hrs.
1	1/8 saturated $CHCl_3$ in H_2O, 44 hrs.	8.5†	11.0	2.5‡	2.5	5.0
2	M/200 chloral hydrate, 44 hrs.	13.4†	18.9	7.4‡	5.5	12.9
3	N/10 NaCl, 44 hrs.	38.4†	59.3	32.4‡	20.9	53.3
4	N/10 KCl, 46 hrs.	53.6	76.2	47.6	22.6	70.2
5	1/8 saturated $CHCl_3$ in H_2O & N/10 NaCl, 44 hrs.	46.2	61.6	40.2	15.4	55.6
6	1/8 saturated $CHCl_3$ & N/10 KCl, 46 hrs.	47.2	76.5	41.2	29.3	70.5
7	M/200 chloral hydrate & N/10 NaCl, 46 hrs.	49.1	79.3	43.1	30.2	73.3
8	M/200 chloral hydrate & N/10 KCl, 46 hrs.	60.5	84.2	54.5	23.7	78.2
9	N/10 NaCl & N/10 KCl 46 hrs.	70.7	82.7	64.7	12.0	76.7
10	N/20 NaCl & N/20 KCl, 46 hrs.	41.8	71.7	35.8	29.9	65.7
11	Control (dist. H_2O renewed every 2 days)	11.4	10.8	5.4	−.6	4.8
12	Control (dist. H_2O not renewed)	16.7	25.0	10.7	8.3	19.0

* All readings represent values of x on the Wheatstone bridge with a resistance in the box of 9,110 ohms.

† Reading taken after 50 minutes. ‡ Increase in first 50 minutes.

‖ The average reading of the distilled water before placing roots in it was approximately 6.0.

Merely to get a basis of comparison between the effects produced by the various agents above mentioned and acid and alkali in certain concentrations, plants were placed in solutions of KOH and H_2SO_4 of approximately the limiting concentrations for root growth, as found by Kahlenberg and True ('96). Instead of excretion being greater than absorption the reverse was found to be true during the period the plants remained in the solutions. The plants, to all external appearances, were not affected adversely in the least, and when later

placed in distilled water gave practically no greater exosmosis than the control. With stronger concentrations a marked effect would undoubtedly be produced. The results obtained are given in table xv. Another point worthy of note in this

TABLE XIV

EFFECTS OF COPPER SULPHATE AND CHLORAL HYDRATE USED SINGLY AND COMBINED UPON THE EXOSMOSIS FROM THE ROOTS OF PLANTS

Cult. no.	Treatment	Specific conductivity of the sol'ns.*			Values of x,† or bridge readings of the Distilled Water				
		Before roots in the sol'n.	After 27 hrs. in the sol'n.	Increase in 27 hrs.	After ½ hr. in the H_2O	After 63 hrs. in the H_2O	Increase the 1st ½ hr. ††	Increase the next 62½ hrs.	Total increase in 63 hrs. ††
1	M/10,000 $CuSO_4$, 28 hrs.....	2.92	16.68	13.76	19.7	55.4	13.7	35.7	49.4
2	M/100 $CuSO_4$, 27 hrs.....	142.40	151.10	8.70	22.0	22.4	16.0	.4	16.4
3	M/8,000 chloral hydrate, remaining to end of exp.....	.35	1.16	.81	20.6‡	13.1**	14.6§	−7.5	7.1**
4	M/100 chloral hydrate, 26 hrs...	.37	3.04	2.67	14.8	16.8	8.8	2.0	10.8
5	M/10,000 $CuSO_4$ and M/8,000 chloral hydrate, 26 hrs.....	2.82	19.77	16.95	15.0	53.3	9.0	38.3	47.3
6	M/100 $CuSO_4$ and M/100 chloral hydrate, 26 hrs.	82.69	95.25	12.56	17.2	20.2	11.2	3.0	14.2
7	Control (dist. H_2O, changed every 4 days).....	.32	.79	.47	15.0‖	12.9††	9.0§	−2.1	6.9††
8	Control (dist. H_2O, not changed)...	.....	.....	.....	14.8	10.7	8.8	−4.1	4.7

* The values given are to be multiplied by 10^{-5} to obtain specific conductivity values.

† Resistance in box 9,110 ohms.

‡ After 26 hours in the solution.

§ The first 26 hours.

‖ After 26 hours in distilled H_2O.

†† The average reading of the distilled water before placing roots in it was approximately 6.0.

** After 89 hours in the solution.

†† After 89 hours in the water.

connection and seen in table xv is the additional verification of the fact that the rinsing method used throughout this investigation was effective and that no electrolytes were carried

over on the roots from the full nutrient solutions, salt solutions, and other media to the distilled water, at least not in sufficient quantity to affect the validity of the results in any way. Although the conductivity of the acid and alkaline media was very high, it is seen by reference to table xv that after rinsing the roots in the usual manner and transferring the cultures to distilled water the readings were very low, thus showing that practically no electrolytes were carried over on the roots.

TABLE XV

CONDUCTIVITY READINGS OF THE CULTURE MEDIA OF PLANTS IN KOH, H_2SO_4, AND LATER IN DISTILLED WATER

Cult. no.	Treatment	Specific conductivity* of the sol'ns.				Values of κ,† or bridge readings of the distilled water				
		Before roots in the sol'n.	After 22 hrs. in the sol'n.	After 47 hrs. in the sol'n.	Increase in 47 hrs.	After ½ hr. in the H_2O	After 50 hrs. in the H_2O	Increase the 1st ½ hr.††	Increase the next 49½ hrs.	Total increase in the 50 hrs.††
1	N/12,800 H_2SO_4, 47 hrs.....	2.39	1.50	1.03	−1.36	10.8	10.5	4.8	−.3	4.5
2	N/6,400 H_2SO_4, 47 hrs.....	5.62	2.53	1.17	−4.45	8.7	9.6	2.7	.9	3.6
3	N/400 KOH, 47 hrs.....	42.02	20.09	16.39	−25.63	11.5	8.7	5.5	−2.8	2.7
4	N/200 KOH, 47 hrs.....	58.50	27.98	24.16	−34.34	11.2	10.5	5.2	−.7	4.5
5	Control (dist. H_2O, not changed)...	.98	2.56	1.32	.34	22.8‡	17.8**	16.8‡	−5.0	11.8**

* The values given are to be multiplied by 10^{-5}.
† The resistance in the box was 9,110 ohms.
‡ After being in distilled water 47 hours.
†† The average reading of the distilled water before placing roots in it was approximately 6.0.
** After 97 hours.

IX. General Discussion

In the foregoing experiments we have been able to note the exosmosis of electrolytes following different treatments. As compared with the controls we have seen marked excretions in some cases and slight or no exosmosis in excess of that in the controls in others. In the normal untreated cultures, or controls, we have seen that there is almost universally a slight exosmosis from the roots into the distilled water for about 24

hours or so, and then in most cases there is a decline in the conductivity curve to a point approaching the original position, after which there may or may not be a gradual incline, depending, probably, on various factors.

It might be well briefly to consider some theoretical aspects of the subject, especially in regard to the causal agencies effecting the increased exosmosis of the treated cultures. The mere transfer of a culture from a full nutrient solution to distilled water is not in itself sufficient to account for the effects produced, as we have seen that osmotic effects play little or no rôle in this connection, a conclusion in harmony with the findings of Loeb ('03) and of True ('14). To what then is the exosmosis due? Can it all be laid at the door of cell cytolysis? What influence has an alteration of the plasma membrane?

In any case, we are dealing with the effect of physical and chemical factors upon the plant cell. For our purpose here it is not considered necessary to enter upon a discussion of the various ideas regarding the details of the structure of the cell and its limiting membrane, or the work and theories of the different investigators on both the animal and plant side concerning the permeability of the plasma membrane. Yet in passing, it may be well to mention Overton's theory regarding the lipoid nature of the plasma membrane, Nathansohn's idea of a mosaic structure of the same, Czapek's experiments indicating the presence of neutral fats in the membrane, Lepeschkin's view that the plasma membrane is a continuous film (some of the work of the last two investigators being summarized by Blackman, '12), and Kite's work on the structure of protoplasm, and also make note of the recent work of Cranner ('14) on the lipoid content of the cell wall.

The effect of the two physical factors, heat and cold, may undoubtedly be considered as resulting in a complete or incipient disorganization of the cell, depending upon the duration of exposure, and a consequent escape of some of the contents into the surrounding medium.

In the case of the various chemical factors or agents used the matter is probably not so simple or so easily disposed of. However, a conception that would fulfill the requirements

theoretically and also accord with the experimental results would seem to be based on the specificity of chemical reaction. The cell, with its complex aggregation of chemical substances, may be considered as interacting with the substance employed, be it anesthetic, toxic agent, salt solution, or other chemical. It may be assumed that each substance has a greater affinity (if we may use that tabooed chemical term) for a particular component of the cell than for other constituents and hence reacts accordingly. This was exemplified by the striking comparison between the effect produced by anesthetics in certain concentrations and that produced by the KCl or NaCl solution. The exosmosis, it is true, was considerable in both cases, but the resulting appearance of the roots was markedly different, the anesthetics causing indications of flaccidity, while the roots exhibiting quite as much exosmosis in the salt solutions, remained practically normal. If we assume that the anesthetic acted upon the colloidal matrix or gel portion of the cell and thus more or less destroyed its organization, while the salts reacted with the substances in the sol condition and left the matrix more or less intact, we would seem to have a basis for explaining the differences observed.

Anesthesia has been considered by Lillie, Osterhout, and others to be essentially a reversible process, provided that the concentration of the anesthetic was not sufficient to be toxic. The experimental work reported herewith, however, on the excretion of electrolytes induced by various anesthetics does not seem to substantiate that view. If the concentration of the anesthetics employed was below a certain point there was no observable effect whatsoever. By increasing the concentration the critical point was attained when excretion began, and as the concentration of the anesthetic was further increased, or as the period of application was lengthened, excretion likewise increased. The excretion process induced by anesthetics therefore conformed in every way to an irreversible chemical reaction. In Osterhout's conductivity measurements of tissue, secondary agglutinization phenomena may possibly have entered in to give the observed effects, and thus have masked the real chemical reaction. Recovery of organ-

isms after anesthetic treatment has also been considered by some as evidence indicating the reversibility of the anesthetic action. If such be viewed from the standpoint of chemical reactions, however, the mere fact of recovery of the organism to a normal condition following the application of anesthetics would not seem to be sufficient justification for concluding that the chemical reaction which initiated the effect is a reversible one, especially when one considers the manifold activities of the cell and the wonderful recuperative powers possessed by organisms, these no doubt involving numerous reactions. Hence the writer is inclined to the belief that an irreversible chemical reaction was at the basis of the phenomena observed as a result of the treatment of the plant with anesthetics and the consequent exosmosis of substances contained in the cell, and that any alteration of the plasma membrane resulting in changed permeability finds its best explanation on the basis of actual chemical reactions.

It is further believed that the results obtained by antagonistic pairs of salts and by single salts are also to be explained, as far as resulting exosmosis is concerned, in the specificity of the action of each. The method employed herein gives a delicate register of such action and is considered to be especially desirable because in it growth phenomena, with their resulting complex nutritive relations, may be left out of consideration. That the high conductivity readings in the case of the salts and certain other electrolytes was not due to insufficiency of the washing before the roots were placed in the distilled water was abundantly proved in various ways.

In regard to the method of experimentation employed in the work here reported, mention may well be made of its adaptability for delicate determinations pertaining to the relative toxicity of different substances. In the past such determinations have been made by means of growth measurements. It would seem that in this method we have, in some respects, a more rapid and satisfactory procedure for such work.

X. Summary and Conclusions

A brief historical review is given of the subject of excretion

from plant roots, exosmosis from living cells, and of excretion from leaves and other tissues.

The methods of experimentation are described.

A theoretical discussion is given of the various aspects of the subject.

The following are some of the experimental results obtained:

(a) Pea seedlings grew better in distilled water in which exosmosis from the previously treated plants of the first crop had occurred than in fresh distilled water, or in distilled water in which untreated plants had been grown.

(b) Peas and horse beans did not do as well in distilled water in which pea seedlings had already grown for 21 days as in fresh distilled water.

(c) Abundant exosmosis may occur from treated plants, even though the roots remain entirely normal in appearance. When the tops were badly affected and the roots remained normal, abundant exosmosis also occurred and the indications pointed in some cases to a downward flow of substances into the roots and out into the aqueous medium. No conclusive proof of this was obtained, however.

(d) Anesthetic vapors cause marked exosmosis upon considerable exposure of the plants to them, but there is none if the exposure be short. The interval required to initiate exosmosis was accurately determined. The order of effectiveness of the vapors tried is, ether, least; illuminating gas, more; and chloroform, most.

(e) The time limits for the exposure of plants to extremes of temperature in relation to exosmosis were determined. Comparison was also made between the effect of dry and moist heat.

(f) The exosmosis curves for various organic compounds were found. In general, at the concentrations used, marked excretion was produced.

(g) The effects of single salts, salts in pairs, and salts plus anesthetics in solution were ascertained as regards the exosmosis produced upon the plants in such solutions. Antagonistic relations in the sense of one substance decreasing the

exosmotic effect produced by another substance were found not to hold in the cases tried and under the conditions of the experiment.

It is with pleasure that the writer acknowledges his indebtedness to Dr. B. M. Duggar for numerous helpful suggestions in the prosecution of this work, and to Mrs. Amy Lyman Merrill for valuable assistance in the calculations involved and in the plotting of the curves; also to Dr. J. R. Schramm, who kindly aided in the tedious work of preparing the manuscript for the printer.

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MONOGRAPH OF THE NORTH AND CENTRAL AMERICAN SPECIES OF THE GENUS SENECIO—PART II[1]

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Introduction

The study upon which this monograph is based was begun nearly twenty years ago, at which time the author was an Assistant at the Gray Herbarium of Harvard University. Nearly every collection of any considerable size which came to the Herbarium, particularly from western United States, Mexico, and Central America, contained specimens of *Senecio,* many of which were either undetermined or referred doubtfully to some obscure or little known species. The identification of such material was often a laborious task, since all species recorded from a given region had to be listed and then specific identity established by a process of elimination. The available publications for such work were De Candolle's 'Prodromus,' Gray's 'Synoptical Flora,' and Hemsley's splendid contribution to the systematic literature of the botany of Mexico and Central America in the 'Biologia Centrali-Americana'; but the results obtained were often very unsatisfactory, because of the large number of new species published in scattered papers during the two decades following the appearance of the 'Synoptical Flora' and the 'Biologia.'

It was felt, therefore, that a revision of the genus, in the light of recent and more complete collections, which have accumulated from the numerous botanical explorations in different parts of North America, would be helpful to those concerned with this difficult group of plants and especially in the organization of material in different herbaria. A critical study of *Senecio* with the view of publishing eventually a

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monograph was suggested to me by Dr. B. L. Robinson, Curator of the Gray Herbarium, who very kindly offered to place at my disposal the entire representation of this genus in the Gray Herbarium, and who, moreover, willingly granted me the exceptional privilege of taking abroad the North American specimens, including all the types, for comparison and study in European herbaria. Accordingly nearly 2,000 mounted specimens were taken to Berlin; and through the courtesy of the authorities of the Royal Botanical Gardens and Museums of Berlin every facility in that institution, which is remarkably rich in Central and South American plants, was accorded me and work on the task was begun under the direction of Professor A. Engler.

It was necessary first of all to acquire a detailed knowledge of the general morphology of the genus *Senecio* as a whole, and also of the closely allied genera. The results of these investigations are briefly recorded in the first part of this monograph, namely 'Monographie der nord- und central-amerikanischen Arten der Gattung Senecio, I. Teil' which is frequently referred to in the following text. This preliminary work and the rich collections of the Gray and Berlin Herbaria form, therefore, the basis for the present systematic part of the monograph.

After completing my studies in Berlin I went to London, taking the Gray Herbarium specimens with me, and there spent several weeks, particularly in the examination of authentic and type specimens at the Kew Herbarium and in the Linnean Herbarium. The opportunity at Berlin, Kew, and Paris to actually compare side by side and in detail, recent specimens, or series of specimens, with many of the older types, some of which are more or less incomplete, has been of very great advantage, and, in fact, has made it possible to establish beyond doubt the identity of many of our American species.

In addition to those herbaria mentioned it also has been my good fortune to study this group of plants in several American institutions, notably the Herbarium of the Geological Survey of Canada, the United States National Her-

barium, the New York Botanical Garden Herbarium (including the Torrey Herbarium), the Herbarium of the Field Museum of Natural History, the Herbarium of the Philadelphia Academy of Natural Sciences, the Missouri Botanical Garden Herbarium, and a number of private collections. To the directors and curators of all these, as well as the owners of the private herbaria, and correspondents who have facilitated my work, I wish to express personal thanks; but I desire especially to extend most grateful acknowledgments to Dr. Benjamin Lincoln Robinson, Asa Gray Professor of Systematic Botany at Harvard University, and Geheimrath Professor Dr. Adolph Engler, Director of the Royal Botanical Gardens and Museum of Berlin, without whose coöperative interest and extreme liberality in the use of valuable scientific material under their charge, this work would have been impossible. I am also grateful to Mr. W. Botting Hemsley, of the Kew Herbarium, through whose courtesy I secured type material of certain rare Mexican species and a number of excellent drawings, some of which are herein reproduced.

I have cited exsiccatae rather freely, particularly such as occur in American herbaria, but by no means all that have been examined, and I have given even at the expense of much repetition detailed citation of specimens in different herbaria, hoping that this would be helpful in the interpretation of species and to future students of the genus. The few plates which it is possible to include are chosen to illustrate more especially the different sections as here defined.

SENECIO [TOURN.] LINN.

Senecio [Tourn. Inst. 456. *pl. 260.* 1700] L. Sp. Pl. **2**: 866. 1753; Gen. Pl., ed. 5, 373, n. 857. 1754; Hill, Hort. Kew. 25. 1768, and ed. 2, 1769; Juss. Gen. Pl. 181. 1789; Less. Syn. 391. 1832; DC. Prodr. **6**: 340. 1837; Endl. Gen. 458, n. 2811. 1838; Hook. Fl. Bor. Am. **1**: 331. 1840, in part; Torr. & Gray, Fl. N. Am. **2**: 436. 1843; Benth. & Hook. f. Gen. Pl. **2**: 446. 1873, in part; Pfeiffer, Nom. Bot. 2^2: 1136. 1874; Hemsl. Biol. Cent.-Am. Bot. **2**: 235. 1881, excl. *Cacalia;* Gray, Syn. Fl. N. Am. 1^2: 383. 1884, and ed. 2, 1888; Hoffmann in Engl. & Prantl,

Nat. Pflanzenf. IV. Abt. 5, 296. 1892, excl. *Emilia;* Greenm. Monogr. Senecio, I. Teil, 1901, and in Engl. Bot. Jahrb. **32** : 1–33. 1902; Dalla Torre & Harms, Gen. Siph. 563. 1900–1907, mainly.

Jacobaea Thunb. Fl. Cap. Prodr. Praef. 1794.

Obaejaca Cass. Dict. Sci. Nat. **35** : 270. 1825.

Roldana LaLlave & Lex. Nov. Veg., fasc. 2, 13. 1825.

Rugelia Schuttlew. in Chapm. Fl. Southern U. S. 246. 1860.

Cacalia, Cineraria, and *Gynoxis,* in part, of authors.

Heads heterogamous and radiate, or discoid. Involucre cylindrical campanulate, occasionally flask-shaped, usually subtended by calyculate bracteoles; bracts of the involucre uniseriate, or by overlapping subbiseriate, variable in number but tending to approach a definite series of numbers, namely 5–8–13–21. Ray-flowers when present disposed in a single row, fertile; rays sometimes more or less reduced. Disk-flowers perfect; corollas slenderly tubular to abruptly ampliated above into a campanulate 5-toothed limb, teeth mostly short. Anthers obtuse or slightly sagittate at the base. Style-branches subterete, recurved-spreading, truncate, rounded-obtuse, occasionally terminated by a small penicillate tuft of hairs, or (in the subgenus *Pseudogynoxis*) terminated by a triangular acute or acuminate appendage. Achenes subterete, usually ribbed, glabrous, or more or less hirtellous especially on the ribs. Pappus of numerous usually white setae.—Annual, biennial, or perennial herbs, shrubs, climbers, or even arboreous plants, with alternate or radical, very variable, pinnately or palmately veined, entire or variously divided leaves.

Synopsis of the Subgenera and Sections

Subgenus I. Eusenecio Hoffm. Style-branches truncate, rounded-obtuse or occasionally terminated by a penicillate tuft of hairs.

A. Stems erect or ascending, not climbing.
  a. Stems not abruptly terminated by a foreshortening of the main axis; oil-tubes not richly developed in the peripheral portion of the stem.
    α. Leaves pinnately veined; lateral nerves not numerous or conspicuous.
      I. Annual herbs .....§ 1. *Annui*

II. Biennial or perennial herbs (rarely annual).

1. Stems herbaceous.

* Heads usually radiate; flowers yellow, except in *S. Greenei* and *S. crocatus*.

† Stem leafy to the inflorescence; leaves laciniately pinnatifid to triternately divided.

0. Native species .....§ 2. *Eremophili*

00. Introduced species ...§ 3. *Jacobaeae*

†† Stem not uniformly leafy to the inflorescence; leaves pinnate or the lower simple and undivided.

0. Leaves pinnate or pinnatisect, rarely undivided .....§ 4. *Sanguisorboidei*

00. Lower leaves rotund-ovate, simple and undivided .....§ 5. *Bolanderiani*

††† Stem not uniformly leafy to the inflorescence; leaves simple and entire to lyrate-pinnatifid; plants either quite glabrous from the start or more or less permanently tomentose; pubescence never of long jointed hairs.

0. Plants glabrous or early glabrate; leaves upwardly reduced on the stem..§ 6. *Aurei*

00. Plants at first tomentose, later glabrate; leaves more uniform throughout and mostly pinnately divided .....§ 7. *Lobati*

000. Plants permanently tomentose or more or less glabrate; stem-leaves upwardly reduced .....§ 8. *Tomentosi*

†††† Stem leafy to the inflorescence (except in § 9); pubescence usually of long jointed hairs.

0. Stem-leaves not amplexicaul.

δ. Leaves not digitately divided ..§ 9. *Columbiani*

δδ. Leaves digitately divided .....§ 10. *Digitati*

00. Stem-leaves amplexicaul.

δ. Involucre ecalyculate .....§ 11. *Cineraroidei*

δδ. Involucre calyculate .....§ 12. *Amplectentes*

	** Heads discoid; flowers whitish or purplish.		
	† Heads 2 cm. or more high; corollas deeply 5-lobed	§ 13.	*Rugeliae*
	†† Heads 1 cm. high; corollas shortly 5-toothed	§ 14.	*Mulgedifolii*
	2. Stems ligneous at the base.		
	* Involucre barely calyculate; plants densely white-tomentose throughout	§ 15.	*Incani*
	** Involucre calyculate; plants glabrous or pubescent	§ 16.	*Suffruticosi*
	3. Shrubs or tree-like plants	§ 17.	*Fruticosi*
β.	Leaves palmately veined	§ 18.	*Palmatinerves*
γ.	Leaves pinnately veined; lateral nerves parallel-arcuate, numerous and conspicuous	§ 19.	*Multinervii*
b.	Stems abruptly terminated by a fore-shortening of the main axis and bearing at the top two to several, more or less pedunculate axillary compound corymbose cymes; oil-tubes richly developed in the peripheral portion of the stem	§ 20.	*Terminales*
B.	Stems climbing	§ 21.	*Streptothamni*

Subgenus II. PSEUDOGYNOXIS Greenm. Style-branches terminated by triangular acute or acuminate dorsally hispidulous appendages § 22. *Convolvuloidei*

SUBGENUS I. EUSENECIO Hoffm.

Subgenus I. EUSENECIO Hoffm. in Engl. & Prantl, Nat. Pflanzenf. IV. Abt. 5. 297. 1892; Greenm. Monogr. Senecio, I. Teil, 21, 30. 1901, and in Engl. Bot. Jahrb. 32 : 17, 26. 1902.

Annuals, biennials or perennials; stems erect, scandent or climbing; leaves pinnately or palmately veined; heads radiate or discoid; style-branches truncate or rounded-obtuse, not infrequently bearing a penicillate tuft of hairs at the extreme tip. Sect. 1–21.

SECT. 1. ANNUI Hoffm.

§ 1. ANNUI Hoffm. in Engl. & Prantl, Nat. Pflanzenf. IV. Abt. 5, 297. 1892; Greenm. Monogr. Senecio, I. Teil, 21, 23. 1901, and in Engl. Bot. Jahrb. 32 : 17, 19. 1902. *Obaejacae* DC. Prodr. 6 : 341. 1837.

Annual herbs; heads radiate or discoid; involucre narrowly campanulate or subcylindric, usually calyculate; achenes pubescent or glabrous. Sp. 1–7.

Key to the Species

A. Heads radiate or discoid; rays when present minute, barely surpassing the involucre.
  a. Plants viscid-pubescent ..... 1. *S. viscosus*
  b. Plants glabrous or pubescent, not viscid.
    α. Leaves coarsely dentate, auriculate-clasping by a broad base ..... 2. *S. mohavensis*
    β. Leaves chiefly pinnatifid, not greatly expanded at the base.
      I. Bracteoles black-tipped, heads discoid ..... 3. *S. vulgaris*
      II. Bracteoles not black-tipped; heads minutely radiate.
        1. Plants slightly pubescent ..... 4. *S. sylvaticus*
        2. Plants glabrous ..... 5. *S. aphanactis*

B. Heads radiate; rays conspicuous, much surpassing the involucre.
  a. Plants glabrous or pubescent, not arachnoid-tomentose.
    α. Leaves thin ..... 6. *S. californicus*
    β. Leaves thickish, succulent ..... 6a. var. *ammophilus*
  b. Plants arachnoid-tomentose ..... 7. *S. ampullaceus*

1. Senecio viscosus L. Sp. Pl. **2** : 868. 1753, and ed. 2, 1217. 1763; Sow. Eng. Bot. *pl. 32*. 1790; Willd. Sp. Pl. **3** : 1984. 1800; Oeder, Fl. Dan. *pl. 1230*. 1799; Schkuhr, Handb. *pl. 267*. 1808; DC. Prodr. **6** : 342. 1837; Gray, Syn. Fl. N. Am. 1^2 : 394. 1884; Greenm. Monogr. Senecio, I. Teil, 23. 1901, in Engl. Bot. Jahrb. **32** : 19. 1902, and in Gray, Manual, ed. 7, 853. 1907; Britton, Manual, ed. 2, 1029. 1905; Britton & Brown, Ill. Fl., ed. 2, **3** : 540. 1913.

Obaejaca viscosa Cass. Dict. Sci. Nat. **35** : 270. 1825.

A strong-scented annual, viscid-pubescent throughout; stem erect, 2 to 4 dm. high, usually branched from the base; leaves sessile, half-clasping, 3 to 6 cm. long, two-thirds as broad, once or twice pinnatifid with angulate-sinuate lobes and rounded sinuses; heads radiate (rarely discoid); rays inconspicuous; achenes glabrous.

Distribution: eastern North America from Nova Scotia to Pennsylvania, near the coast.

Specimens examined:

Nova Scotia: Pictou, 1 Nov., 1874, *Fowler* (Field Mus. Herb.); Pictou Landing, 21 July, 1883, *Macoun 14883* (Geol. Surv. Canada Herb.); Kentville, 22 Aug., 1902, *Fernald* (Gray Herb. and Geol. Surv. Canada Herb.).

New Brunswick: Shediac, 11 Sept., 1874, *Fowler* (Geol.

Surv. Canada Herb. *14882* and Kew Herb. *872*, in part); Painsec Junction, 8 Aug., 1901, *Churchill* (Gray Herb.).

Massachusetts: along Boston and Albany Railroad, Sept., 1879, *Boott* (Gray Herb.); streets of Cambridge, 1 Sept., 1897, *Robinson* (Gray Herb.).

Rhode Island: wharves at Providence, 4 Sept., 1874, *Congdon* (Gray Herb.); streets of Providence, coll. of 1876, *Bailey* (Gray Herb. and Field Mus. Herb.); East Providence, 20 July, 1890, *Collins* (Mo. Bot. Gard. Herb.).

Pennsylvania: on ballast, Girard Point, July, 1877, *Martindale* (Gray Herb.) and Aug., 1877, *Rothrock* (Field Mus. Herb.). Introduced from Europe.

2. S. mohavensis Gray, Syn. Fl. N. Am. 1^2: 446. 1884, and ed. 2, 454. 1886; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32**: 19. 1902. Plate 17.

Glabrous throughout; stems erect or nearly so, 1.5 to 4 dm. high, freely branching; leaves membranous, ovate to oblong-ovate, 2 to 6 cm. long, 1 to 4 cm. broad, apiculate-acute, irregularly toothed, or somewhat laciniate-dentate, the lowermost narrowed into a petiolate base, those of the stem sessile and amplexicaul; inflorescence a terminal corymbose cyme; heads 1 cm. high on slender peduncles, discoid or with much reduced ligulate flowers; involucre calyculate with few short inconspicuous bracteoles, 18–20-flowered; bracts of the involucre about 13, linear, acute, slightly shorter than the flowers of the disk; achenes canescent pubescent.

Distribution: southern California, Arizona, and northern Mexico.

Specimens examined:

California: Pleasant Cañon, Panamint Mountains, alt. 900 m., 10 May, 1906, *Hall & Chandler 6910* (Mo. Bot. Gard. Herb. and Field Mus. Herb.); Hall Cañon, Panamint Mountains, 18 April, 1891, *Coville & Funston 697* (U. S. Nat. Herb.); Panamint Valley, alt. 450 m., 5 May, 1897, *Jones* (Mo. Bot. Gard. Herb.); Mohave region, April–May, 1884, *Lemmon 3129* (Gray Herb.), TYPE; Colorado Desert, April, 1889, *C. R. Orcutt* (U. S. Nat. Herb. and Gray Herb.).

Arizona: Tempe, 21 April, 1892, *Ganong & Blaschka* (Gray Herb.).

Sonora: near the U. S. boundary line, 28 March, 1884, *Pringle* (Gray Herb. and U. S. Nat. Herb.).

3. S. vulgaris L. Sp. Pl. **2**: 867. 1753, ed. 2, 1216. 1763; Fl. Dan. *pl. 513.* 1770; Willd. Sp. Pl. **3**: 1979. 1800; Sow. Eng. Bot. *pl. 747.* 1800; Pursh, Fl. **2**: 528. 1814; DC. Prodr. **6**: 341. 1837; Reichb. Ic. Fl. Germ. & Helv. **16**: 35. *pl. 68* (CMLIX), *fig. I, 1–9.* 1854; Gray, Syn. Fl. N. Am. 1^2: 394. 1886; Greenm. Monogr. Senecio, I. Teil, 23. 1901, in Engl. Bot. Jahrb. **32**: 19. 1902, and in Gray, Manual, ed. 7, 853. 1907; Britton, Manual, ed. 2, 1029. 1905; Britton & Brown, Ill. Fl., ed. 2, **3**: 539. 1913.

Annual, 1 to 4 dm. high, glabrous or subfloccose pubescent especially in the axils of the upper leaves and in the inflorescence; leaves 2 to 8 cm. long, 0.5 to 2.5 cm. broad, more or less lyrately pinnatifid and angulate-toothed, lower leaves narrowed into a margined petiole, the upper sessile and semiamplexicaul; heads discoid; the rather numerous small calyculate bracteoles as well as the bracts of the involucre usually black-tipped; achenes hirtellous-puberulent along the angles or ribs.

Distribution: Labrador, Newfoundland to North Carolina, west to Alaska, California, and New Mexico. Europe, Asia, and Africa.

Specimens examined:

Labrador: Hopedale, 4–6 Aug., 1897, *Sornborger 162* (Gray Herb.).

Newfoundland: rocky hills, St. John's, 1 Aug., 1894, *Robinson & Schrenk* (Gray Herb., U. S. Nat. Herb., Geol. Surv. Canada Herb., and Mo. Bot. Gard. Herb.); Funk Island, 23 July, 1887, *Palmer* (U. S. Nat. Herb.); rich soil, field near shore, Channel, 27 July–1 Aug., 1901, *Howe & Lang 802* (Gray Herb.); Barred Island, 13 Aug., 1903, *Sornborger* (Gray Herb.).

Nova Scotia: dry soil, roadsides, North Sydney, Cape Breton, 21–25 July, 1901, *Howe & Lang 639* (Gray Herb.);

Boylston, July, 1890, *Hamilton 22848* (Geol. Surv. Canada Herb.); Baddeck, Cape Breton Island, 25 July, 1898, *Macoun 19721* (Geol. Surv. Canada Herb.).

New Brunswick: along railroad, Conners, 22 July, 1908, *Mackenzie 3646* (Mo. Bot. Gard. Herb.); Shediac, 11 Sept., 1874, *Fowler 872* in part. (Kew Herb.).

Quebec: shore of St. Lawrence, Gaspé, Matane Co., *Forbes* (Gray Herb.); Gaspé Basin, 24 July, 1882, *Macoun 14889* (Geol. Surv. Canada Herb.).

Ontario: Ottawa, 20 July, 1891, *Scott 14885* (Geol. Surv. Canada Herb.); Belleville, 10 Aug., 1877, *Macoun 14890* (Geol. Surv. Canada Herb.); northeast of Sarnia, Lambton Co., *Wheatley* (Mo. Bot. Gard. Herb.); Wingham, Aug., 1890, *Morton 14886* (Geol. Surv. Canada Herb.); Kingston, Sept., 1896, *Fowler* (Field Mus. Herb.); Sarnia, 18 June, 1901, *Macoun 26677* (Geol. Surv. Canada Herb.).

Saskatchewan: between Cumberland House and Hudson Bay, *Richardson 14887* (Geol. Surv. Canada Herb.); Prince Albert, 13 July, 1896, *Macoun 12174* (Geol. Surv. Canada Herb.).

Alberta: waste ground, Prince's Island, near Calgary, 21 Aug., 1913, *Moodie 31* (Field Mus. Herb.).

British Columbia: Burrard Inlet, 22 July, 1889, *Macoun* (Gray Herb. and Geol. Surv. Canada Herb.); vicinity of Victoria, 9 April, 1908, *Macoun 78949* (Field Mus. Herb.); along railway embankment, Sicamous, 20 July, 1904, *Macoun 62191* (Geol. Surv. Canada Herb.); Cedar Hill, Vancouver Island, 21 May, 1887, *Macoun 14884* (Geol. Surv. Canada Herb.); near Victoria, 23 May, 1893, collector not indicated, *550* (Geol. Surv. Canada Herb.); Victoria, 10 June, 1875, *Dawson 14888* (Geol. Surv. Canada Herb.).

Alaska: vicinity of Sitka, July, 1891, *Wright 1538* (Mo. Bot. Gard. Herb.); Sitka, July, 1881, *McLean* (U. S. Nat. Herb.); Skagway, 29 July, 1907, *Coulter 889* (Field Mus. Herb. and Mo. Bot. Gard. Herb.).

Maine: Baker's Island, 19 July, 1883, *Redfield* (Mo. Bot. Gard. Herb.).

Vermont: waste ground, Rutland, 1 Sept., 1899, *Eggleston 1383* (Gray Herb.).

Massachusetts: Ipswich, *Oakes* (Gray Herb. and U. S. Nat. Herb.); Nahant, 6 July, 1878, *Kellermann* (Mo. Bot. Gard. Herb.); Revere Beach, 9 July, 1898, *Greenman 515* (Gray Herb.); Cambridge, *Chickering* (U. S. Nat. Herb.); roadsides, West Cambridge, 29 Sept., 1894, local collection (Gray Herb.); Swampscott, 21 June, 1897, *Weatherby* (Gray Herb.); Ipswich, July, 1874, *Morong* (Field Mus. Herb.).

Rhode Island: waste places, Providence, Sept., 1844, *Thurber* (Gray Herb.); Providence, 2 July, 1892, *Collins & Bailey* (U. S. Nat. Herb.); Cat Swamp, Providence, 23 June, 1895, *Collins* (U. S. Nat. Herb.); Providence, 16 Aug., 1873, *Congdon* (Field Mus. Herb.); Providence, July, 1878, *Bailey* (Mo. Bot. Gard. Herb.).

New York: Syracuse, June, 1887, *Overacker* (Mo. Bot. Gard. Herb.); Troy, collector and date not indicated (Gray Herb.); Ithaca, 12 Oct., 1892, *H. von Schrenk* (Mo. Bot. Gard. Herb.); near Fiske mansion, Ithaca, 21 May, 1884 (U. S. Nat. Herb.); Hunter's Point, Long Island, Sept., 1879, *J. Schrenk* (U. S. Nat. Herb.); Elmira City, 28 Aug., 1898, *Lucy* (Field Mus. Herb.); Troy, June, 1873, *Jesup* (Field Mus. Herb.).

Pennsylvania: Girard Point, Philadelphia, Aug., 1877, *Rothrock* (Field Mus. Herb.).

New Jersey: Camden, July, 1876, *Martindale* (U. S. Nat. Herb.); Kaighn's Point, Camden, 16 July, 1865, *Parker* (Mo. Bot. Gard. Herb.).

Maryland: vicinity of Oakland, 5 Sept., 1910, *Steele* (U. S. Nat. Herb.).

District of Columbia: waste ground, Washington, 14 Sept., 1891, *Blanchard* (Mo. Bot. Gard. Herb.); above Uniontown, 27 May, 1883, *Ward* (U. S. Nat. Herb.).

North Carolina: cultivated grounds, Biltmore, 4 May, 1897, *Biltmore Herb. 883*^{b} (Gray Herb., Mo. Bot. Gard. Herb., and Field Mus. Herb.).

Ohio: Oberlin, June, 1892 and 1895, *Ricksecker* (U. S. Nat. Herb.).

Michigan: waste ground, Keweenaw Co., July, 1887, *Farwell* (Gray Herb.).

Wisconsin: St. Croix Co., coll. of 1888, *Matthews* (U. S. Nat. Herb.); Preble, 20 May, 1883, *Schuette* (Field Mus. Herb.); Green Bay, 11 July, 1897 and 29 Sept., 1901, *Schuette* (Field Mus. Herb.).

Nebraska: Valley Co., July, 1886, *Webber* (Field Mus. Herb.).

Montana: Willow Creek, 14 June, 1883 *Scribner 123*[c] (Gray Herb.); Columbia Falls, 21 June, 1894, *Williams 965* (Gray Herb. and U. S. Nat. Herb.).

Wyoming: Sundance, 4 July, 1896, *Nelson 2201* (Mo. Bot. Gard. Herb.).

Colorado: valley near Empire, Sept., 1892, *Patterson* (Gray Herb.); along railroad at Georgetown, Aug.–Sept., 1892, *Patterson* (Field Mus. Herb.).

New Mexico: Sante Fe, 14 Sept., 1895, *Mulford 1301* (Mo. Bot. Gard. Herb.); 4 May, 1897, *A. A. & E. G. Heller 3657* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.).

Idaho: frequent in moist places, Julietta, Latah Co., 8 June, 1892, *Sandberg, McDougal & Heller 343* (Gray Herb., Field Mus. Herb., and U. S. Nat. Herb.); waste ground in the Palouse Country and about Lake Coeur d'Alene, June–July, 1892, *Aiton* (Field Mus. Herb. and Mo. Bot. Gard. Herb.).

Washington: on mountains near the lower Cascades, 29 May, 1886, *Suksdorf* (Gray Herb.); Seattle, 6 March, 1889, *Smith* (Mo. Bot. Gard. Herb.); in fields, Pullman, 2 June, 1894, *Piper* (Mo. Bot. Gard. Herb.); Hoquiam, 5 June, 1897, *Lamb 1146* (Field Mus. Herb. and Mo. Bot. Gard. Herb.); San Juan Island, July, 1914, *Reynolds* (Field Mus. Herb.); Index, Snohomish Co., July, 1898, *Savage, Cameron & Lenocker* (Field Mus. Herb.); Granddalles, 3 Sept., 1904, *Westgate 3997* (U. S. Nat. Herb.); Klickitat Co., June, 1878, *Suksdorf* (Gray Herb.).

Oregon: cultivated fields, Sauvie Island, June, 1880, *Howell* (Gray Herb.); Portland, 1 June, 1884, *Henderson 555* (Mo. Bot. Gard. Herb.); Portland, Feb., 1900, *Lunell*, and without date *Sargent* (Gray Herb.); Bonneville, 6 Aug., 1895,

Canby (U. S. Nat Herb.); Catching Inlet, 10 May, 1911, *Smith 3700* (Field Mus. Herb.); Charleston Bay, 6 May, 1911, *Smith 3668* (Field Mus. Herb.); North Slough, 1 March, 1911, *Smith 3487;* Coos Co., 2 March, 1911, *Smith 3494* (Field Mus. Herb.); Portland, March, 1889, *Drake & Dickson* (Field Mus. Herb.); without definite locality, coll. of 1868–69, *Kellogg & Harford 536* (U. S. Nat. Herb.).

California: Oakland, March, 1864, *Bolander 2777* (Gray Herb. and Mo. Bot. Gard. Herb.) and May, 1865, *Bolander 434* (Gray Herb.); without definite locality, coll. of 1880, *Norton* (Mo. Bot. Gard. Herb.); near Mendocino, May, 1898, *Brown 758* (Mo. Bot. Gard. Herb.); Mendocino Co., June, 1898, *Brown 458* (Field Mus. Herb.); Stanford University, 2 March, 1902, *Baker 311* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); Russian River, near Trenton, 16 March, 1902, *Heller & Brown 5072* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); Big River, Mendocino Co., July, 1903, *McMurphy 374* (U. S. Nat. Herb.); near Saratoga, Santa Clara Co., 25 Feb., 1906, *Pendleton 288* (U. S. Nat. Herb.).

4. S. sylvaticus L. Sp. Pl. **2**:868. 1753, and ed. 2, 1217. 1763; Sow. Eng. Bot. *pl. 748.* 1800; Willd. Sp. Pl. **3**:1985. 1800; Fl. Dan. *pl. 869.* 1782; DC. Prodr. **6**: 342. 1837; Gray, Syn. Fl. N. Am. 1^2: 394. 1884; Greenm. Monogr. Senecio, I. Teil, 23. 1901, in Engl. Bot. Jahrb. **32**: 19. 1902, and in Gray, Manual, ed. 7, 853. 1907; Britton, Manual, ed. 2, 1029. 1905; Britton & Brown, Ill. Fl., ed. 2, **3**: 539. 1913.

Obaejaca sylvatica Cass. Dict. Sci. Nat. **35**: 271. 1825.

Stem erect, simple or branched, 1 to 4 dm. or more high, usually somewhat pubescent; leaves more or less pinnatifid with unequal lobes, 2 to 15 cm. long, 1 to 8 cm. broad; the lower leaves petioled, the upper sessile, clasping and auriculate-sagittate; inflorescence naked or nearly so; heads cylindrical, sparingly calyculate, radiate; ligules barely surpassing the involucre, not infrequently much reduced; achenes canescent-pubescent.

Distribution: Newfoundland to Maine, Ohio, and on Pacific coast.

Specimens examined:

Newfoundland: railway ballast, Whitbourne, 17 Aug., 1894, *Robinson & Schrenk* (Gray Herb., U. S. Nat. Herb., Mo. Bot. Gard. Herb., and Geol. Surv. Canada Herb.).

Prince Edward Island: sand dunes, Tracadie Beach, 25 July, 1901, *Churchill* (Gray Herb. and Mo. Bot. Gard. Herb.); waste places, Brackley Point, 28 Aug., 1888; *Macoun 14874* (Geol. Surv. Canada Herb.).

Nova Scotia: clearings and open woods, Sydney, Cape Breton Island, 17 Aug., 1902, *Fernald* (Gray Herb.); Boylston, Aug., 1890, *Hamilton 22847* (Geol. Surv. Canada Herb.); Truro, without date, *Macculloch* (Gray Herb.); Elizabethtown, Cape Breton Island, 2 Aug., 1898, *Macoun 19719* (Gray Herb. and Geol. Surv. Canada Herb.); Baddeck Bay, Cape Breton Island, 11 Aug., 1898, *Macoun 19720* (Gray Herb. and Geol. Surv. Canada Herb.); sea cliffs, Black Hole, near Baxter's Harbor, 24 Aug., 1902, *Fernald* (Gray Herb.); on pebbly beach, Purcell's Cove, Halifax Harbor, 2–6 Sept., 1901, *Howe & Lang 1512* (Gray Herb.); open woods, Starrs Point, Kings Co., 23 Aug., 1902, *Fernald* (Gray Herb.); McNiels Harbor, Cape Breton Island, 4 Aug., 1898, sheet *19722* (Geol. Surv. Canada Herb.).

New Brunswick: Grand Manan, 26 July, 1891, *Churchill* (Field Mus. Herb. and Mo. Bot. Gard. Herb.); Falls of the St. John River, St. John, 22 July, 1902, *Williams & Fernald* (Gray Herb.).

Quebec: beach of Gaspé Bay, Gaspé Co., 24–27 Aug., 1904, *Collins, Fernald & Pease* (Gray Herb.).

British Columbia: Vancouver Island, 6 Aug., 1909, *Macoun 78950* and *78951* (Field Mus. Herb.).

Maine: island in Penobscot Bay, Aug., 1896, *F. L. & L. H. Harvey 554*[c] (U. S. Nat. Herb.).

Ohio: near Painsville, coll. of 1892, *Hacker 123* (Gray Herb.).

Washington: Seattle, Aug., 1909, *Piper* (U. S. Nat. Herb.); on old burn near farms, Port Crescent, Aug., 1911, *Webster 19* (U. S. Nat. Herb.); old camps, Granite Falls, Snohomish Co., 31 Oct., 1911, *Smith 4226* (Field Mus. Herb.); Iron Mountain,

Granite Falls, alt. 300 m., 28 Oct., 1911, *Smith 4224* (Field Mus. Herb.).

Oregon: region of Coos Bay, 10 Sept., 1911, *House 4848* (U. S. Nat. Herb.).

California: Vance's Camp, Humboldt Co., 5 June, 1911, *Smith 3778* (Field Mus. Herb.); vicinity of Eureka, 20 June, 1907, *J. P. Tracy 2571* (Univ. Calif. Herb. and Mo. Bot. Gard. Herb.).

5. **S. aphanactis** Greene, Pittonia, **1**: 220. 1888, and Fl. Franciscana 464. 1897; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32**: 19. 1902.

S. sylvaticus Gray, Bot. Calif. **1**: 410. 1876, not L.; Jepson, Fl. West. Mid. Calif. 512. 1901.

A slender annual, 1 to 3 dm. high, glabrous or somewhat tomentulose especially in the inflorescence; stem simple or branched; leaves linear to lanceolate, 1 to 4 cm. long, 1 to 12 mm. broad, entire to coarsely dentate or even pinnately lobed, glabrous or nearly so; the lower leaves narrowed into a petiolate base, the upper sessile; inflorescence terminal, few to several-headed; heads somewhat flask-shaped, 6 to 7 mm. high, radiate; involucre sparingly bracteolate, glabrous to tomentulose at the base; rays small, scarcely exceeding the involucre; achenes appressed-canescent.

Distribution: central California, northern Mexico and adjacent islands.

Specimens examined:

California: Mare Island, 30 March, 1874, *Greene* (Gray Herb. and Field Mus. Herb.), CO-TYPE; San Luis Obispo, *Brewer 463* (Gray Herb. and Mo. Bot. Gard. Herb.); San Luis Obispo, coll. of 1886, *Summis* (U. S. Nat. Herb.); Avalon, Santa Catalina Island, March, 1901, *Trask* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); edges of cañons and alkaline flats, San Diego, *Brandegee 3414* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); San Diego, Geological Survey of California 1860–61, *Cooper* (Gray Herb.); North American Pacific coast flora, *Parry 170* (Gray Herb.); San Diego, 5 Feb., 1884, *Orcutt* (Field Mus. Herb.).

Lower California: Cedros Island, April, 1897, *Brandegee* (Gray Herb. and U. S. Nat. Herb.); San Quentin Bay, *Palmer 606* (Kew Herb.).

6. **S. californicus** DC. Prodr. 6 : 426. 1837; Torr. & Gray, Fl. N. Am. 2 : 437. 1843; Gray, Bot. Calif. 1 : 410. 1876, Syn. Fl. N. Am. 1^2 : 393. 1884, and ed. 2, 454. 1886; Greene, Fl. Franciscana, 465. 1897; Greenm. Monogr. Senecio, I. Teil, 23. 1901 and in Engl. Bot. Jahrb. 32 : 19. 1902; Abrams, Fl. Los Angeles and vicinity 439. 1904.

S. californicus var. *laxior* DC. Prodr. 6 : 426. 1837; Torr. & Gray, Fl. N. Am. 2 : 437. 1843.

S. coronopus Nutt. Trans. Am. Phil. Soc. 7 : 413. 1841; Torr. & Gray, Fl. N. Am. 2 : 437. 1843.

An herbaceous glabrous annual; stem erect simple or branched, 1 to 5 dm. high; leaves oblong-spatulate to lanceolate, entire to subpinnatifid, 2.5 to 7 cm. long, .2 to 2 cm. broad, often reddish; the lower leaves often narrowed to a subpetiolate base, the upper sessile and auriculate-clasping at the base; heads radiate, few to several in a loose cyme; bracts of the involucre about 21, often brownish or black-tipped, much exceeded by the yellow conspicuous rays; achenes canescent-pubescent.

Distribution: central California, vicinity of Monterey, south to northern Mexico.

Specimens examined:

California: sand hills, back of Seaside, Monterey Co., 3 April, 1903, *Heller 6509* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); Gigling Station, east of Del Monte, in sand, 11 May, 1903, *Heller 6710* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); Bardins, Monterey Co., April, 1903, *Elmer 4893* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Del Monte, April, 1902, *Elmer 3576* (Mo. Bot. Gard. Herb.); Arroja Grande, San Luis Obispo Co., 21 Feb., 1886, *Summers* (Gray Herb.); Cuyama, near the boundary between Santa Barbara and San Luis Obispo Counties, 6 May, 1896, *Eastwood* (Gray Herb.); hillsides, Los Angeles Co., 19 March, 1888, *Hasse* (U. S. Nat. Herb.); Los Angeles, May,

1888, *Hasse* (Field Mus. Herb.); copses and grassy slopes, Los Angeles Co., May, 1890, *Hasse* (U. S. Nat. Herb.); Santa Monica, coll. of 1885, *A. Gray* (Gray Herb.); hillsides, Los Angeles Co., Aug., 1890 and June, 1891, *Hasse* (Mo. Bot. Gard. Herb.); "Pueblo los Angeles," *Gambell* (Gray Herb.); without definite locality, *Coulter 335* (Gray Herb.), and coll. of Nov., 1846, *Fremont* (Gray Herb.); Los Angeles, 5 April, 1890, *Fritchey* (Mo. Bot. Gard. Herb.); San Bernardino, *S. B. & W. F. Parish 198* (Field Mus. Herb. and Mo. Bot. Gard. Herb.); San Bernardino, coll. of 1880, *Vasey 330* (Field Mus. Herb., U. S. Nat. Herb., and Gray Herb.); near San Bernardino, May, 1893, *Parish* (Mo. Bot. Gard. Herb.); mesas, San Bernardino Co., May, 1888, *Parish* (Mo. Bot. Gard. Herb.) and April, 1896, *Parish* (Field Mus. Herb.); San Bernardino Co., coll. of 1876, *Parry & Lemmon 206* (Field Mus. Herb. and Mo. Bot. Gard. Herb.); Arrow Head Springs, 15 May, 1891, *Fritchey 18* (Mo. Bot. Gard. Herb.); San Bernardino, *Parish 7* (Gray Herb.); "Cocomurgo," in sandy places, March, 1854, *Bigelow* (Gray Herb.); San Bernardino Co., Feb.–April, 1882, *Parish 233* (Gray Herb.); without definite locality, coll. of 1833, *Douglas 46* (Gray Herb.), CO-TYPE of var. *laxior;* vicinity of Riverside, alt. 600 m., March, 1903, *Hall 3721* (Gray Herb. and Field Mus. Herb.); San Diego, April, 1873, *Bolander & Kellock* (Gray Herb.); San Luis Rey, *Parry* (Gray Herb.); vicinity of Riverside, 26 March, 1907, *Reed 1252* (Field Mus. Herb.); vicinity of San Bernardino, 13 April, 1903, *Parish 5188* (Field Mus. Herb.); without definite locality, *Nuttall* (Gray Herb.); San Diego, April, 1905, *Brandegee* (U. S. Nat. Herb.) and April, 1902, *Brandegee 1647* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); San Diego, April, 1882, *Jones* (U. S. Nat. Herb.); hills, San Diego, 25 April, 1882, *Pringle* (U. S. Nat. Herb. and Field Mus. Herb.); San Diego, 4 May, 1882, *Orcutt 328* (Mo. Bot. Gard. Herb.); Potrero, 6 April, 1889, *Orcutt* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Moro hills, near Fallbrook, 28 April, 1903, *Abrams 3332* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Fallbrook, 27 March, 1882, *Jones 3118* (U. S. Nat. Herb.); San Diego, *Cleveland* (Field

Mus. Herb. and Mo. Bot. Gard. Herb.); San Diego, coll. of June, 1906, *K. Brandegee* (U. S. Nat. Herb.), and coll. of 1875, *Palmer 200* (Field Mus. Herb. and Mo. Bot. Gard. Herb.); side hill, Del Mar, Oct., 1894 and 22 March, 1895, *Angier 14* and *97* (Mo. Bot. Gard. Herb.); Mesa, April, 1895, *Angier* (Field Mus. Herb.); La Jolla, San Diego Co., 17 Feb., 1895, *Snyder* (Field Mus. Herb.); Las Paderes Ranch, San Diego Co., 26 Feb., 1888, *Deane* (Field Mus. Herb.).

Lower California:

Todos Santos Bay, July, 1883, *Orcutt 708* (Gray Herb.); All Saints Bay, May, 1882, *Fish* (Gray Herb.); Punta Bauda, 25 Jan., 1883, *Orcutt 708* (Mo. Bot. Gard. Herb.); Nachoguero Valley, *Schoenfeldt 3401* (U. S. Nat. Herb.).

Var. **ammophilus** (Greene) Greenm. comb. nov.

Senecio ammophilus Greene, Bull. Cal. Acad. 1:193. 1886.

Leaves thickish, somewhat succulent, 2 to 4 cm. long, .2 to 1.5 cm. broad, the lower oblanceolate subentire, those of the stem auriculate-clasping, pinnately lobed into oblong or linear obtuse lobes.

Lower California: Cape San Quentin, 10 May, 1885, *Greene* (Gray Herb.), CO-TYPE.

The thick leaves of this variety give the plant a somewhat different appearance from typical forms of the species; but an examination of a large suite of specimens shows numerous transitional forms such as those secured by *Fritchey, Pringle, Bigelow, Palmer 200, Orcutt 708,* and *K. Brandegee.*

7. **S. ampullaceus** Hook. Bot. Mag. *pl. 3487.* 1836; DC. Prodr. 6: 428. 1836; Torr. & Gray, Fl. N. Am. 2: 440. 1843; Engelm. & Gray, Boston Jour. Nat. Hist. 5: 250. 1845 (Pl. Lindh. 1: 42. 1845); Gray Syn. Fl. N. Am. 1^2: 393. 1884, and ed. 2, 1886; Coulter, Contr. U. S. Nat. Herb. 2: 241. 1892; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. 32: 19. 1902.

S. ampullaceus var. *glaberrimus* Engelm. & Gray, Boston Jour. Nat. Hist. 5: 250. 1845 (Pl. Lindh. 1: 42. 1845).

S. ampullaceus var. *floccosus* Engelm. & Gray, Boston Jour. Nat. Hist. 5: 250. 1845 (Pl. Lindh. 1: 42. 1845).

Annual, or occasionally becoming biennial, more or less floccose-tomentose throughout, somewhat glabrate; leaves oblong-obovate, acute to lanceolate and acuminate, 5 to 18 cm. long, 1 to 7 cm. broad, entire to coarsely and irregularly dentate; the lower leaves narrowed below into a winged petiole, those of the stem sessile, semiamplexicaul, gradually smaller towards the few to many headed cymose inflorescence; heads 10 to 12 mm. high, radiate, including the rays 1.5 to 3 cm. in diameter; involucre setaceous-calyculate; bracts of the involucre glabrous; achenes pubescent.

Distribution: eastern Texas.

Specimens examined:

Texas: San Felipe, Austin Co., *Drummond* (Kew Herb. and Gray Herb.), TYPE; Corsicana, *Reverchon* (Mo. Bot. Gard. Herb.); near Richland Station, 13 March, 1880, *Joor* (Mo. Bot. Gard. Herb.); Dawson, 16 April, 1903, *Reverchon 3965* and *5965* (Mo. Bot. Gard. Herb.); Llano, May, 1885, *Reverchon 1545* (U. S. Nat. Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); banks of Pecan Bayou, April, 1882, *Reverchon 81* (Gray Herb. and Mo. Bot. Gard. Herb.); sandy soils, Lampasas Co., May, 1884, *Reverchon 1321* (Mo. Bot. Gard. Herb.); Crabapple, Gillespie Co., *Jermy* (Mo. Bot. Gard. Herb.); Hockley, Harris Co., coll. of 1890, *Thurrow* (Field Mus. Herb.); banks of Colorado River, 4 April, 1914, *Young* (Mo. Bot. Gard. Herb. and Univ. of Texas Herb.); on dry ground, Hempstead, 24 April, 1872, *Hall 369* (Gray Herb., U. S. Nat. Herb., and Mo. Bot. Gard Herb.); without locality, coll. of 1848, *Wright* (Gray Herb.); Industry, Austin Co., coll. of 1890, *Wurzlow* (Field Mus. Herb.); banks of railroad near Rosenberg, Fort Bend Co., 13 April, 1900, *Eggert* (Mo. Bot. Gard. Herb.); common on prairies, Columbia, 10 April, 1899, *Bush 95* (Gray Herb. and Mo. Bot. Gard. Herb.); Columbia, 23 April, 1900, *Bush 122* (Mo. Bot. Gard. Herb.); Columbia, 25 March, 1900, *Canby, Sargent & Trelease 153* (U. S. Nat. Herb.); on moist prairie between the Brazos and the Colorado Rivers, April, 1844, *Lindheimer 268, 269* (Mo. Bot. Gard. Herb.), CO-TYPES of var. *glaberrimus* and *floccosus*.

Sect. 2. Eremophili Greenm.

§ 2. Eremophili Greenm. Monogr. Senecio, I. Teil, 21, 23. 1901, and in Engl. Bot. Jahrb. **32** : 17, 19. 1902.

Annual or biennial herbs, not infrequently becoming perennial by the development of a ligneous base; stems leafy; leaves laciniately pinnatifid; inflorescence a terminal corymbose or paniculate cyme; heads radiate, rays conspicuous; achenes glabrous or pubescent. Sp. 8–13.

Key to the Species

A. Plants glabrous; achenes smooth or slightly hirtellous.
  a. Heads 7 to 10 mm. high; involucral bracts 5 to 7 mm. long, usually conspicuously black-tipped.
    α. Involucre 3 to 5 mm. in diameter, 20–35-flowered ..... 8. *S. MacDougalii*
    β. Involucre 5 to 6 mm. in diameter, 35–50-flowered ..... 9. *S. ambrosioides*
  b. Heads 10 to 12 mm. high; involucral bracts 7 to 10 mm. long, not conspicuously black-tipped.
    α. Northern species (Canada and the U. S.) ..... 10. *S. eremophilus*
    β. Southern species (Mexico) ..... 11. *S. Townsendii*

B. Plants more or less tomentose; achenes canescent-pubescent.
  a. Leaves at first tomentulose, later glabrate ..... 12. *S. chihuahuensis*
  b. Leaves permanently tomentulose ..... 13. *S. durangensis*

8. **S. MacDougalii** Heller, Bull. Torr. Bot. Club **26** : 592. 1899; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32** : 19. 1902, in part; Rydb. in Fl. Colo. 397. 1906, in part; Wooton & Standley, Contr. U. S. Nat. Herb. **19** : 745. 1915.

S. eremophilus Gray, Syn. Fl. N. Am. 1[2] : 392. 1884, and ed. 2, 1886, in part, not Richards.

S. eremophilus var. *attenuatus* Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32** : 19. 1902.

Glabrous throughout or slightly puberulent above; stem simple or branched, 5 to 8 dm. high, leafy to the inflorescence; leaves more or less laciniately pinnatifid, 3 to 10 cm. long, 1.5 to 5 cm. broad, segments linear to lanceolate, entire to coarsely and unequally dentate; inflorescence terminating the stem and branches in corymbose cymes; heads 7 to 10 mm. high, radiate; involucre narrowly campanulate, calyculate, 3 to 5 mm. in diameter; bracts of the involucre usually 13 (8–13), linear-

lanceolate, 4 to 5 mm. long, commonly black-tipped; ray-flowers 5 to 8, light yellow; disk-flowers 14 to 30; achenes glabrous or slightly puberulent.

Distribution: New Mexico and Arizona.

Specimens examined:

New Mexico: Santa Fe Cañon, Aug., 1880, *Snow* (Mo. Bot. Gard. Herb.); Santa Fe Creek, 9 Sept., 1881, *Engelmann* (Mo. Bot. Gard. Herb.); Santa Fe, 14 Aug., 1895, *Mulford 1292* (Mo. Bot. Gard. Herb.); near Pecos, alt. 2040 m., 25 Aug., 1908, *Standley 5311* (Mo. Bot. Gard. Herb.); Pecos River National Forest, alt. 2560 m., 10 Aug., 1908, *Standley 4873* (U. S. Nat. Herb.); White Mountains, alt. 2130 m., 6 Aug., 1897, *Wooton 290* (Gray Herb. and Mo. Bot. Gard. Herb.); White Mountains, alt. 2255 m., 25 Aug., 1907, *Wooton & Standley 3672* (U. S. Nat. Herb.); head of Bear Creek, coll. of 1903, *Plummer* (U. S. Nat. Herb.); Gilmore's Ranch, White Mountains, alt. 2280 m., 23 Sept., 1906, *Standley* (Mo. Bot. Gard. Herb.); G. O. S. Ranch, Grant Co., 27 Aug.–12 Sept., 1911, *Holzinger* (U. S. Nat. Herb.).

Arizona: Walnut Cañon, alt. 2130 m., *MacDougal 342* (Gray Herb. and Field Mus. Herb.), CO-TYPE; near Flagstaff, May–Oct., 1900, *Purpus* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Mt. Agassiz, alt. 3050 m., 10 Sept., 1909, *Pearson 315* (U. S. Nat. Herb.); Humphrey Peak, July, 1883, *Rusby 337* (Gray Herb. and Field Mus. Herb.); Barfoot Park, Chiricahua Mountains, 24 Oct., 1906, *Blumer 1484* (U. S. Nat. Herb. and Field Mus. Herb.); Huachuca Mountains, Sept., 1882, *Lemmon 2785* (Gray Herb., U. S. Nat. Herb., and Field Mus. Herb.); Huachuca Mountains, 17 Oct., 1903, *Mearns 2581* (U. S. Nat. Herb.).

9. **S. ambrosioides** Rydb. Bull. Torr. Bot. Club **37**: 467. 1910; Wooton & Standley, Contr. U. S. Nat. Herb. **19**: 745. 1915.

S. eremophilus Gray, Pl. Fendl. 108. 1849, as to plant of Fendler; Pac. Rail. Rept. **4**: 111. 1856, as to plant of Bigelow; Syn. Fl. N. Am. 1^2: 392. 1884, and ed. 2. 1886, in part, not

Richards.; Nelson, in Coulter & Nelson, Manual Cent. Rocky Mountains, 583. 1909, in part, not Richards.

S. MacDougalii Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. 32 : 19. 1902, in part, not Heller; Rydb. Fl. Colo. 397. 1906, in part, not Heller.

Herbaceous perennial, glabrous or essentially so throughout; stems one to several from a ligneous base, 3 to 5 dm. high; leaves oblanceolate to ovate-lanceolate in general outline, 3 to 13 cm. long, 1 to 5 cm. wide, more or less laciniately pinnatifid into linear to lanceolate, entire to coarsely and unequally dentate divisions; inflorescence a terminal corymbose cyme; heads usually numerous, 7 to 10 mm. high, radiate; involucre subcampanulate, 5 to 7 mm. in diameter, calyculate; bracts of the involucre usually 13, linear-lanceolate, 5 to 7 mm. long, commonly black-tipped; ray-flowers 5 to 8; disk-flowers 30 to 45; achenes hirtellous-puberulent.

Distribution: Wyoming to New Mexico, Idaho, and Arizona.

Specimens examined:

Wyoming: gravelly banks, Centennial Mountain, Albany Co., 2 Aug., 1902, *Nelson 8773* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); moist ground in open woods, Centennial, 27 July, 1900, *Nelson 7717* (Gray Herb. and Mo. Bot. Gard. Herb.); Bridger Peak, Carbon Co., 22 Aug., 1903, *Goodding 1942* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Colorado: Chamber's Lake, alt. 2895 m., 13 Sept., 1896, *Baker* (Mo. Bot. Gard. Herb.); cañon west of Palmer Lake, alt. 2435 m., 12 Aug., 1896, *Crandall* (Mo. Bot. Gard. Herb.); Steamboat Springs, 20 July, 1903, *Goodding 1617* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Boulder, coll. of 1908, *Pace* (Mo. Bot. Gard. Herb.); Denver, 8 Sept., 1905, *Moffat* (Mo. Bot. Gard. Herb.); Georgetown, 19 Aug., 1895, *Shear 4720* (U. S. Nat. Herb.), coll. of 19 July, 1886, *Trelease*, and coll. of 26 July, 1886, *Letterman* (Mo. Bot. Gard. Herb.); Rocky Mountains, Powell's Colorado Exploring Expedition 1868, *Vasey 337* (Gray Herb.); Golden City, 18 Aug., 1870, *Greene 230* (Gray Herb.); Silver Plume, 21 Aug., 1895, *Shear 4999* (U. S. Nat. Herb.); Manitou, Aug., 1881, *Fritchey 14*, in part, and coll. of 16 Aug., 1884, *Letterman* (Mo. Bot. Gard. Herb.);

Ruxton Park, alt. 2700 m., 21 Aug., 1901, *F. E. & E. S. Clements 152* (Gray Herb., U. S. Nat. Herb., and Mo. Bot. Gard. Herb.); Breckenridge, coll. of 1887, *Bereman* (Mo. Bot. Gard. Herb.); Breckenridge, coll. of 1892, *Wislizenus 1063* (Mo. Bot. Gard. Herb.); Oro City, 23 July, 1873, Hayden's U. S. Geol. Survey, *Coulter,* in part (U. S. Nat. Herb.); Green Mountain Falls, alt. 2560 m., 2 Aug., 1892, *Sheldon 485* (U. S. Nat. Herb.); Hotchkiss, alt. 1585 m., 30 June, 1892, *Cowen 287* (U. S. Nat. Herb.); Oak Creek, Fremont Co., Aug., 1873, *Brandegee 716* (Mo. Bot. Gard. Herb.); Gunnison, 25 July, 1901, alt. 2300 m., *Baker 596* (Gray Herb. and Mo. Bot. Gard. Herb.); vicinity of Mount Carbon, Gunnison Co., alt. 2730–2800 m., 4 July and 10 Aug., 1910, *Eggleston 5835* and *6159* (U. S. Nat. Herb.); Pandora, 10 Aug., 1901, *Baker 748* (Gray Herb. and Mo. Bot. Gard. Herb.); Taylor River, 15 Aug., 1873, Hayden's U. S. Geol. Survey, *Coulter* (U. S. Nat. Herb.); Telluride, alt. 2740–3600 m., Aug., 1894, *Tweedy 354* (U. S. Nat. Herb.); Ute Pass, 2 July, 1896, *Shear 3695* (U. S. Nat. Herb.); near Pagosa Peak, alt. 3050 m., 8 Aug., 1899, *Baker 706* (Gray Herb., U. S. Nat. Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); Saguache Creek, Sept., 1873, Wheeler Expedition, *Wolf 1086* (U. S. Nat. Herb.); Parrott City, alt. 2740 m., *Baker, Earle & Tracy 475* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); northeast corner of North Park, 3 Aug., 1874, *Barber* (U. S. Nat. Herb.); Twin Lakes, Wheeler Expedition, 1873, *Wolf & Rothrock 562* (Gray Herb., and Field Mus. Herb.); Rocky Mountains, coll. of 1862, *Hall & Harbour 327* (Gray Herb. and Field Mus. Herb.), also coll. of 1861–62, *Parry 26* (Gray Herb. and Mo. Bot. Gard. Herb.); mouth of Bear Creek Cañon, 23 Aug., 1915, *Drushel & Dougan* (Drushel Herb.); upper Clear Creek Valley, alt. 3050 m., 10 Aug., 1874, *Engelmann* (Mo. Bot. Gard. Herb.); Leadville, 8 July, 1886, *Trelease* (Mo. Bot. Gard. Herb.); Tolland, alt. 2895 m., 29 July, 1913, *Overholts* (Mo. Bot. Gard. Herb.); near Breckenridge, alt. 2950 m., Aug., 1901, *Mackenzie 208* (Mo. Bot. Gard. Herb.); Penn's Gulch, near Sunset, 30 July, 1886, *Letterman* (Mo. Bot. Gard. Herb.).

New Mexico: pine forest, Jicarilla Apache Reservation,

near Dulce, alt. 2150–2470 m., 20 Aug., 1911, *Standley 8183* (U. S. Nat. Herb.); Chama, 8 Sept., 1899, *Baker* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Santa Fe Cañon, 3 Oct., 2380–2850 m., 8 July, 1911, *Standley 6564* (U. S. Nat. Herb.); Navajo Indian Reservation in the Tunitcha Mountains, 8 Aug., 1911, *Standley 7591* (U. S. Nat. Herb.); mountains near Las Vegas, July, 1881, *Vasey* (U. S. Nat. Herb.); Santa Fe Cañon, 7 July, 1897, alt. 2440 m., *A. A. & E. G. Heller 3819* (Gray Herb. and Mo. Bot. Gard. Herb.); Sante Fe Cañon, 3 Oct., 1913, *Rose, Fitch & Parkhurst 17714* (U. S. Nat. Herb.); Canoncinto, Santa Fe Co., coll. of 1879, *Brandegee 12078* (Mo. Bot. Gard. Herb.); creek bottom, Santa Fe, 20 Oct., 1846, *Fendler 475* (Gray Herb. and Mo. Bot. Gard. Herb.); Balsam Park, Sandia Mountains, alt. 2500 m., Aug.–Sept., 1914, *Ellis 281* (Mo. Bot. Gard. Herb.); Pecos River Indian Reservation, 6 Aug., 1898, *Coghill 144* (Mo. Bot. Gard. Herb.); Mineral Creek, Sierra Co., alt. 2130 m., 26 Sept., 1904, *Metcalfe 1415* (U. S. Nat. Herb.); Santa Antonita, Whipple's Exploration 1853–54, *Bigelow* (U. S. Nat. Herb. and Gray Herb.); Organ Mountains, alt. 2130 m., 23 Sept., 1906, *Wooton & Standley* (U. S. Nat. Herb.).

Utah: Big Cottonwood Cañon, Salt Lake Co., alt. 2774 m., 10 Aug., 1905, *Garrett 1591* (U. S. Nat. Herb.); Tate Mine, Marysvale, alt. 2740 m., 22 Aug., 1894, *Jones 5858* (Mo. Bot. Gard. Herb.); Bromide Pass, Henry Mountains, alt. 3050 m., 27 July, 1894, *Jones 5695bd* (U. S. Nat. Herb.); slope of Aquarius Plateau, alt. 2750 m., 2 Aug., 1875, *Ward 499* (U. S. Nat. Herb.).

Arizona: Navajo Indian Reservation, about the north end of the Carrizo Mountains, 29 July, 1911, *Standley 7376* (U. S. Nat. Herb.).

Among the specimens here cited, a few, particularly Parry's 26, Overholts', Mackenzie's 208, and Engelmann's plant from Upper Clear Creek Valley, might be almost equally well referred to the preceding species, *S. MacDougalii*, to which *S. ambrosioides* is very closely related; but in general the latter may be distinguished by the slightly larger and more numer-

ously flowered heads and usually, but not always, less pinnatisect leaves.

10. **S. eremophilus** Richards. in App. Frankl. 1st Journ. 31. 1823; Hook. Fl. Bor. Am. **1** : 334. 1840; Torr. & Gray, Fl. N. Am. **2** : 444. 1843; Eaton, Bot. King Exp. 191. 1871, in part; Gray, Syn. Fl. N. Am. 1^2 : 392. 1884, and ed. 2, 1886; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32** : 19. 1902; Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains 583. 1909, in part.

S. pembrinensis Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32** : 19. 1902.

An herbaceous perennial, glabrous or slightly puberulent in the inflorescence; stems erect, 3 to 8 dm. high, striate; leaves more or less laciniately pinnatifid with linear, lanceolate or oblong, entire or coarsely and unequally dentate divisions; the lower leaves petiolate, the upper subsessile; inflorescence terminating the stem in a somewhat leafy corymbose or paniculate cyme; heads rather large, 10 to 12 mm. high, radiate; involucre campanulate conspicuously calyculate; bracts of the involucre usually 13, linear-lanceolate, acute, 7 to 9 mm. long, glabrous, minutely brownish- or black-tipped; ray-flowers 8 to 10; disk-flowers 40 to 60; achenes ribbed, glabrous, or slightly hirtellous-puberulent.

Distribution: northwestern Canada to Nebraska, Colorado, and Utah.

Specimens examined:

Saskatchewan: Lipton, 11 Aug., 1911, *Clokey 1844* (Mo. Bot. Gard. Herb.); Qu'Appelle River, Assiniboia, Aug., 1883, *Macoun 14839* (Geol. Surv. Canada Herb. and U. S. Nat. Herb.); near Prince Albert, 10 July, 1896, *Macoun 12171* (Geol. Surv. Canada Herb.); in damp thickets north of Saskatchewan River, 22 Aug., 1872, *Macoun 14841* (Geol. Surv. Canada Herb.); Saskatchewan Plains, *Macoun 868* (Kew Herb.).

Alberta: "on gravelly banks of Cedar Lake, Lat. 54°," *Richardson* (Kew Herb.), TYPE; Pembina, coll. of 1873, *Coues* (Gray Herb.); on damp banks, Bow River at Morley, 6 Sept.,

1879, *Macoun 14840* (Geol. Surv. Canada Herb.); Dunvegan, Peace River, 17 Aug., 1879, *Dawson 26686* (Geol. Surv. Canada Herb.); Athabasca Plains, 14 Sept., 1872, *Macoun 1040* (Gray Herb. and Kew Herb.).

South Dakota: Sylvan Lake, 27 Aug., 1897, *Griffiths* (Mo. Bot. Gard. Herb.).

Nebraska: mountain range, south of White Clay Creek, 23 Aug., 1859, Lieut. F. T. Bryan's Expedition, 1856, *H. Engelmann* (Mo. Bot. Gard. Herb.).

Wyoming: on the summits of Big Horn Mountains, Aug., 1859, Reynolds' Expedition to the headwaters of the Missouri and Yellowstone Rivers, *Hayden* (Mo. Bot. Gard. Herb.); Laramie Mountains, *Hayden* (Gray Herb. and Mo. Bot. Gard. Herb.); Laramie Mountains, 17 Aug., 1899, *Schuchut* (U. S. Nat. Herb.).

Colorado: Cascade Cañon, July, 1880, *Eurney* (Mo. Bot. Gard. Herb.); Rocky Mountains, *Hall & Harbour 327*, in part (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Pike's Peak, alt. 3050 m., 25 Aug., 1915, *Drushel & Dougan* (Drushel Herb.); Manitou, Aug., 1881, *Fritchey 14* in part (Mo. Bot. Gard. Herb.), form.

var. **Kingii** (Rydb.) Greenm. comb. nov.

Senecio Kingii Rydb. Bull. Torr. Bot. Club **37**: 468. 1910.

S. eremophilus Eaton, Bot. King Exp. 191. 1871, as to plant of Watson.

S. Watsoni Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32**: 19. 1902.

Leaves oblanceolate to oblong-lanceolate, coarsely dentate to pinnatisect with relatively broad divisions; but through several specimens connecting directly with the above species.

Specimen examined:

Utah: Cottonwood Cañon, alt. 2590 m., Aug., 1869, *Watson 676* (Columbia Univ. Herb. and Gray Herb.), TYPE.

11. S. Townsendii Greenm.[1]

Herbaceous perennial, glabrous throughout; stem 6 to 10 dm. high, striate, often purplish; leaves coarsely, unequally

[1] Senecio Townsendii Greenm. sp. nov., herbaceus perennis ubique glabrus; caule 6–10 dm. alto, striato saepe purpurascenti; foliis inaequaliter et remote

and remotely dentate to laciniately pinnatifid, oblanceolate to oblong-lanceolate in general outline, 3 to 10 cm. long, 1 to 4 cm. broad, divisions linear and entire to dentate, acute or obtuse; lower leaves petiolate, the upper sessile; inflorescence a loose several to many-headed corymbose cyme; heads 10 to 13 mm. high, radiate; involucre narrowly campanulate, calyculate, glabrous; bracts of the involucre commonly 13, linear-lanceolate, 8 to 10 mm. long, terminated by a small black or brownish penicillate tip; flowers pale yellow; ray-flowers 5 to 8, occasionally much reduced; disk-flowers 35 to 50; achenes glabrous.

Distribution: northern Mexico.

Chihuahua: near Colonia San Garcia in the Sierra Madre, alt. 2285 m., 9 Sept., 1899, *Townsend & Barber 317* (Mo. Bot. Gard. Herb., Gray Herb., and U. S. Nat. Herb.), TYPE; Mound Valley, Sierra Madre Mountains, alt. 2130 m., 18 Sept., 1903, *Jones* (U. S. Nat. Herb.).

The Townsend and Barber specimens have been distributed as "*Senecio Chihuahuanus* Wats." and the Jones plant was distributed as "*Senecio eremophilus*" under which names they may be looked for in herbaria.

12. S. chihuahuensis Watson, Proc. Am. Acad. **23**: 280. 1888; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32**: 19. 1902.

An herbacous perennial; stem erect, 4 to 5 dm. high from a rather slender rootstock, striate-angulate, somewhat purplish; early leaves oblanceolate, 3 to 5 cm. long, 1 cm. broad, laciniately dentate, arachnoid-tomentulose on both surfaces; later stem-leaves short-petiolate, or subsessile, oblong-ovate in general outline, about 8 cm. long, one-half to two-thirds as

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grosse-dentatis vel laciniato-pinnatis, oblanceolatis vel oblongo-lanceolatis in circumscriptione, 3–10 cm. longis, 1–4 cm. latis; laciniis linearibus et integris vel dentatis acutis vel obtusis; foliis inferioribus petiolatis, superioribus sessilibus; inflorescentibus laxe corymboso-cymosis multicapitatis; capitulis 10–13 mm. altis, radiatis; involucris anguste campanulatis calyculatis glabris; bracteis involucri 13 lineari-lanceolatis 8–10 mm. longis minute atro- vel fulvo-penicillatis; floribus pallide aurantiabus; floribus femineis 5–8 nonnunquam multo reductis; floribus disci 35–50; achaeniis striatis glabris.—Near Colonia San Garcia in the Sierra Madre, State of Chihuahua, Mexico, alt. 2285 m., 9 Sept., 1899, *Townsend & Barber 317* (Mo. Bot. Gard. Herb., Gray Herb., and U. S. Nat. Herb.), TYPE; Mound Valley, Sierra Madre Mountains, alt. 2135 m., 18 Sept., 1903, *Jones* (U. S. Nat. Herb.).

broad, subbipinnate, at first tomentulose, later becoming glabrous or essentially so, divisions narrow, unequal, cartilaginous-apiculate; inflorescence a terminal corymbose cyme; heads 10 to 12 mm. high, radiate; involucre cylindric-campanulate, calyculate with short linear subulate bracteoles; bracts of the involucre 7 to 9 mm. long, brownish- or black-tipped, shorter than the numerous flowers of the disk; ray-flowers about 8; achenes canescent-pubescent.

Distribution: northern Mexico.

Specimens examined:

Chihuahua: ledges of the Sierra Madre, alt. 2955 m., 7 Oct., 1887, *Pringle 1318* (Gray Herb., Kew Herb., and Mo. Bot. Gard. Herb.), TYPE.

13. S. durangensis Greenm. Field Col. Mus. Bot. Ser. 2 : 275. 1907. Plate 18.

S. ctenophyllus Greenm. Proc. Am. Acad. 43 : 20. 1907, not Phil.

An herbaceous annual, or becoming perennial by the development of a ligneous base; stem simple or branched, erect, 3 to 4 dm. high, arachnoid-tomentose; leaves lanceolate, 2 to 9 cm. long, 1 to 2.5 cm. wide, more or less pinnately divided, permanently arachnoid-tomentulose on both surfaces, lower leaves petiolate, upper sessile; inflorescence a terminal tomentulose corymbose cyme; heads numerous, 8 to 10 mm. high, radiate, calyculate; involucre campanulate, glabrous or nearly so; bracts of the involucre 13, linear-lanceolate, 5 to 6 mm. long, minutely black-tipped, penicillate; ray-flowers 5 to 8, ligules pale yellow; disk flowers 20 to 30; achenes canous-hirtellous.

Distribution: northern Mexico.

Specimen examined:

Durango: barranca, below Sandia Station, alt. 2135 m., 15 Oct., 1905, *Pringle 10105* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.), TYPE.

Sect. 3. Jacobaeae DC.

§ 3. Jacobaeae DC. Prodr. 6 : 348. 1837; Hoffm. in Engl. & Prantl, Nat. Pflanzenf. IV, Abt. 5, 297. 1892; Greenm.

Monogr. Senecio, I. Teil, 21, 23. 1901, and in Engl. Bot. Jahrb. **32** : 17, 19. 1902.

Biennial or perennial herbs with lyrate or 2–3-pinnatisect leaves and radiate heads. Sp. 14–16.

Key to the Species

A. Stem and leaves glabrous or nearly so; involucral bracts narrow, about 1 mm. broad; bracteoles usually black-tipped..... *14. S. rupestris*
B. Stems and leaves more or less permanently floccose-tomentulose; involucral bracts 1.5 to 2 mm. broad; bracteoles not black-tipped.
a. Upper stem-leaves once pinnate..... *15. S. erucifolius*
b. Upper stem-leaves 2–3-pinnatisect..... *16. S. Jacobaea*

14. S. rupestris Waldst. & Kit. Descr. et Ic. Pl. Rar. Hung. **2** : 136. *pl. 128.* 1805; Reichb. Ic. Crit. **4** : 28. *pl. 334. fig. 514.* 1826; Strobl, Fl. Admont. **1**: 57. 1881, and in Flora **65**: 478, 479. 1882; von Hayek, Fl. Stierm. **2** : 564. 1913.

S. laciniatus Bert. in Desv. Jour. Bot. **2**: 76. 1813; Amoen. Ital. 102, 408. 1819.

Senecio nebrodensis var. *glabratus* DC. Prodr. **6** : 350. 1837.

Annual or biennial, sometimes becoming perennial, glabrous throughout or slightly pubescent; stem erect, 3 to 6 dm. high, simple or branched, striate; leaves lanceolate to obovate-lanceolate in general outline, 3 to 10 cm. long, 1 to 4 cm. broad, laciniately lobed or subpinnatiscent, thin in texture, the lobes again sharply dentate; the lower leaves narrowed into a subpetiolate base, the upper sessile and semiamplexicaul; inflorescence a terminal corymbose cyme; heads 8 to 10 mm. high, radiate; involucre calyculate with black-tipped bracteoles; bracts of the involucre about 21, linear-lanceolate, acute, 6 to 7 mm. long; ray-flowers about 13; disk-flowers numerous; achenes glabrous or slightly hirtellous.

Distribution: on ballast near Philadelphia. Introduced from Europe.

Specimen examined:

Pennsylvania: on ballast, Philadelphia, July, 1880, *Martindale* (Gray Herb.).

15. S. erucifolius L. Fl. Suecica, ed. 2, 291. 1755; Huds. Fl. Ang. 366. 1798; DC. Prodr. **6** : 351. 1837; Reichb. Ic. Fl.

Germ. & Helv. **16**: 38. *pl. 75* (CMLXVI). *fig. 1.* 1854; Cosson & Saint-Pierre, Fl. Paris, ed. 10, 518. 1861. Beck von Mannagetta, Fl. Nieder-Oesterr. 1221. 1893.

An herbaceous biennial or perennial, more or less floccose-tomentulose throughout and on the stem and lower leaf-surface often intermixed with hirsute hairs; stems erect, 3 to 10 dm. high, simple or branched; leaves lyrately pinnatifid to pinnatisect, 2 to 10 cm. long, 1 to 6 cm. broad, the lobes subentire, blunt, and submucronate to sharply dentate; lowermost leaves narrowed into a subpetiolate base, the upper sessile and semiamplexicaul; inflorescence a terminal few- to many-headed corymbose cyme; heads about 1 cm. high, radiate; involucre campanulate, calyculate; bracts of the involucre usually 13, lanceolate-oblong, 4 to 5 mm. long, glabrous or slightly floccose-tomentulose, with rather broad scarious margins; ray-flowers about 13; disk-flowers numerous, 50 to 60; achenes hirtellous.

Distribution: on ballast near Philadelphia. Introduced from Europe.

Specimens examined:

Pennsylvania: on ballast, Philadelphia, 30 Aug., 1879, *Parker* (Gray Herb.).

New Jersey: on ballast, Kaighn's Point, *Burk* (Field Mus. Herb.).

16. S. Jacobaea L. Sp. Pl. 2: 870. 1753; Willd. Sp. Pl. 3: 1997. 1800; DC. Prodr. 6: 350. 1837; Sm. & Sow. Eng. Bot. **16**: *pl. 1130.* 1803; Schkuhr, Handb. *pl. 267.* 1808; Reichb. Ic. Fl. Germ. & Helv. **16**: 38. *pl. 73* (CMLXIV). *figs. II. 3, 4.* 1854; Gray, Syn. Fl. N. Am. 1²: 383. 1884, and ed. 2, 1886; Britton, Manual, ed. 2, 1029. 1905; Gray, Manual, ed. 7, 853. 1907; Britton & Brown, Ill. Fl., ed. 2, **3**: 542. 1913.

Jacobaea vulgaris Vahl in Fl. Dan. **6**: *pl. 944.* 1787; Gaertn. Fruct. 2: 445. *pl. 170. fig. 1.* 1791. An erect, biennial or perennial herb, 3 dm. or more high, at first usually arachnoid-tomentulose, more or less glabrate; basal leaves petiolate, somewhat lyrate; stem leaves sessile, semiamplexicaul, ovate-oblong in general outline, 3 to 15 cm. long, 1.5 to 7 cm.

broad, 2–3-pinnatisect; inflorescence a terminal corymbose cyme; heads numerous, radiate; achenes pubescent.

Distribution: Newfoundland to New Jersey, occurring along roadsides, in pastures, and on ballast. Introduced from Europe.

Specimens examined:

Newfoundland: roadsides, St. John's, 7–19 Aug., *Robinson & Schrenk* (Gray Herb., U. S. Nat. Herb., Geol. Surv. Canada Herb., and Mo. Bot. Gard. Herb.).

Nova Scotia: L'Ardoire, Cape Breton Island, Aug., 1892, *Faxon* (Gray Herb.); Sydney and Mira Bay, Cape Breton Island, 17 Aug., 1898, *Macoun 19723* (Geol. Surv. Canada Herb.); eastern Nova Scotia, 16 Aug., 1890, *Chickering* (U. S. Nat. Herb.); Boylston, *Hamilton 22844* (Geol. Surv. Canada Herb.); Pictou, 1 Nov., 1874, *Fowler* (Field Mus. Herb.); Pictou Landing, 24 July, 1883, *Macoun 14859* (Geol. Surv. Canada Herb.); pasture, Windsor Junction, 11 July, 1901, *Howe & Lang 427* (Gray Herb.); pasture, near Pictou, 12–18 July, 1901, *Howe & Lang 540* (Gray Herb.).

Prince Edward Island: Tignish, 26 July, 1888, *Macoun, 14858* (Geol. Surv. Canada Herb.); Tracadie Beach, 27 July, 1901, *Churchill* (Gray Herb. and Mo. Bot. Gard. Herb.).

New Brunswick: Miramichi, *Fowler* (Gray Herb. and U. S. Nat. Herb.); near railroad station, Anagance, 19 July, 1901, *Churchill* (Gray Herb. and Mo. Bot. Gard. Herb.).

Quebec: on ballast-filling about fish houses, York, Gaspé Co., 25 Aug., 1904, *Collins, Fernald & Pease* (Gray Herb.).

Ontario: Burlington, 23 Aug., 1883, *Burgess 14857* (Geol. Surv. Canada Herb.).

Pennsylvania: on ballast, July, 1876, *Martindale* (Mo. Bot. Gard. Herb.).

New Jersey: on ballast, Camden, coll. of 1878, *Martindale* (Gray Herb., U. S. Nat. Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); on ballast, Kaighn's Point, *Burk* (Field Mus. Herb.).

Sect. 4. Sanguisorboidei Greenm.

§ 4. Sanguisorboidei Greenm. Monogr. Senecio, I. Teil, 22,

23, 1901, and in Engl. Bot. Jahrb. **32** : 18, 19. 1902. *Lobati* Rydb. Bull. Torr. Bot. Club **27** : 169. 1900, in part.

Annuals, biennials, or perennials, often forming merely a rosette of leaves during the first year; stems erect, 1.5 to 10 dm. high from a distinctly annual root or from a rather stout rootstock; leaves once, twice, or thrice pinnately divided; heads numerous; achenes glabrous or pubescent. Sp. 17–29.

Key to the Species

A. Annuals or biennials.
  a. Bracts of the involucre usually 13, linear-lanceolate, 1 to 2.5 mm. broad ..... 17. *S. sanguisorboides*
  b. Bracts of the involucre usually 21, linear or linear-lanceolate, 0.5 to 1.5 mm. broad.
    α. Lateral leaf-segments not abruptly contracted into a narrow base.
      I. Plants of southeastern United States. 18. *S. glabellus*
      II. Plants of southwestern Texas and northern Mexico ..... 19. *S. Greggii*
    β. Lateral leaf-segments abruptly contracted into a narrow base ..... 20. *S. imparipinnatus*
B. Perennials; upright stem from a horizontal, ascending or suberect rootstock.
  a. Leaves 2–3-pinnatisect; segments narrow .... 21. *S. Millefolium*
  b. Leaves once pinnate; segments narrowly obovate to subreniform.
    α. Heads numerous, small, 5 to 10 mm. high.
      I. Involucral bracts usually 21.
        1. Leaves glabrous; achenes hirtellous. 22. *S. tampicanus*
        2. Leaves pubescent beneath; achenes glabrous ..... 23. *S. hypotrichus*
      II. Involucral bracts usually 13.
        1. Lateral leaf-divisions longer than broad.
          * Midrib glabrous ..... 24. *S. Sanguisorbae*
          ** Midrib floccose-tomentulose .... 25. *S. pinnatisectus*
        2. Lateral leaf-divisions as broad as long ..... 26. *S. coahuilensis*
    β. Heads fewer and larger, 10 to 14 mm. high.
      I. Leaves pinnately divided nearly to the midrib.
        1. Leaf-divisions few, cuneate to reniform ..... 27. *S. leonensis*
        2. Leaf-divisions many, cuneate to linear ..... 28. *S. montereyana*
      II. Leaves pinnately divided slightly more than half-way from margin to midrib ..... 29. *S. zimapanicus*

17. S. sanguisorboides Rydb. Bull. Torr. Bot. Club **27** : 170. 1900; Wooton & Standley, Contr. U. S. Nat. Herb. **19** : 745. 1915.

Annual or biennial, glabrous or slightly white tomentulose in the axils of the leaves; stem 1.5 to 5 dm. high, striate; leaves usually pinnately divided into cuneate to reniform dentate or crenate-dentate divisions, the terminal division ovate-reniform, 1 to 5 cm. broad; basal and lower stem-leaves petiolate and occasionally undivided; upper stem-leaves sessile and amplexicaul; inflorescence a terminal few to several-headed corymbose cyme; heads radiate; involucre campanulate, barely calyculate; bracts of the involucre usually 13 (rarely 16), lanceolate, 6 to 6.5 mm. long, glabrous; ray-flowers 8 to 10; disk-flowers 30 to 50; achenes ribbed, glabrous.

Distribution: mountains of New Mexico.

Specimens examined:

New Mexico: Willow Gulch, Colfax Co., alt. 3050 m., Aug., 1896, *St. John 115* (Gray Herb.); Santa Fe Cañon, 7 July, 1897, alt. 2440 m., *A. A. & E. G. Heller 3820* (Mo. Bot. Gard. Herb.), CO-TYPE; Santa Fe Creek, 22 June, 1847, *Fendler 438* (Mo. Bot. Gard. Herb.); White Mountains, Lincoln Co., alt. 3048 m., 16 Aug., 1897, *Wooton 494* (Mo. Bot. Gard. Herb.); mouth of Pouchuelo Creek, Pecos River National Forest, alt. 2590 m., 30 June, 1908, *Standley 4093* (Mo. Bot. Gard. Herb.); mouth of Mora River, Pecos River National Forest, alt. 2470 m., 7 July, 1908, *Standley 4250* (Mo. Bot. Gard. Herb.); Pecos River Indian Reservation, 17 July, 1898, *Coghill 71* (Mo. Bot. Gard. Herb.).

18. S. glabellus Poir, Dict. **7**: 102. 1806; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32**: 19. 1902; Gray, Manual, 853, ed. 7, 1907; Britton & Brown, Ill. Fl. **3**: 540, ed. 2, 1913.

S. lyratus Michx. Fl. Bor. Am. **2**: 120. 1803, not L.

S. lobatus Pers. Syn. **2**: 436. 1807; Nutt. Gen. **2**: 165. 1818; Elliot, Sk. **2**: 332. 1824; Torr. & Gray, Fl. N. Am. **2**: 437. 1843; Gray, Syn. Fl. N. Am. 1^2: 394. 1884, and ed. 2, 1886, mainly; Chapman, Fl. Southern U. S. 266, ed. 3, 1897; Britton & Brown, Ill. Fl. **3**: 481, ed. 1, 1898; Small, Fl. Southeastern U. S. 1303. 1903, and ed. 2, 1913; Mohr, Contr. U. S. Nat. Herb. **6**: 815. 1901.

S. carolinianus Spreng. Syst. 3 : 559. 1826.

S. densiflorus Martens, Bull. Acad. Roy. Soc. Brux. 8 : 66. 1841.

S. Schweinitzianus Nutt. Trans. Am. Phil. Soc. 7 : 413. 1841.

Annual or biennial, glabrous or slightly tomentulose in the axils of the leaves; stems erect 1 to 10 dm. high, striate; radical leaves petiolate, lyrate, occasionally undivided; those of the stem petiolate or sessile and semiamplexicaul, pinnately divided into rather remote, narrowly cuneate to subreniform unequal divisions; inflorescence a terminal corymbose cyme; heads 6 to 8 mm. high, radiate; ray-flowers 8 to 12; disk-flowers about 50; achenes usually hirtellous-puberulent.

Distribution: North Carolina west to Illinois, Missouri, and South Dakota, south to Florida and eastern Texas. Common on river bottoms and flood-plains.

Specimens examined:

North Carolina: near Wilmington, April, 1888, *McCarthy* (U. S. Nat. Herb.); without locality, *Curtis* (Gray Herb.).

South Carolina: Goose Creek, 19 May, 1885, *A. C. & F. W. Maier* (Gray Herb.); swamps, Summerville, April, 1890, *Taylor* (Field Mus. Herb.).

Georgia: Macon, coll. of 1875, *Curtiss* (U. S. Nat. Herb.); central Georgia, coll. of 1846, *Porter* (Gray Herb.); Butler Island, McIntosh Co., 27 May, 1909, *Smith 2185* (Field Mus. Herb.).

Florida: without locality, *Chapman* (Gray Herb., U. S. Nat. Herb., and Kew Herb.); Fort Orange, 10 April, 1895, *Straub 103* (Gray Herb.); near Chattahooche, *Curtis 1565* (Gray Herb., U. S. Nat. Herb., Kew Herb., and Field Mus. Herb.); River Junction, 19 April, 1898, *Curtis 6370* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Sand Point, 8 April, 1874, *Palmer 301* (Gray Herb., U. S. Nat. Herb., and Mo. Bot. Gard. Herb.); near St. Marks, coll. of 1843, *Rugel* (Mo. Bot. Gard. Herb., and Kew Herb.); Losman's Key, May, 1891, *Simpson 154* (U. S. Nat. Herb. and Field Mus. Herb.); New

Smyrna, *Burgess 563* (Field Mus. Herb.); Gulf Hammock, April, 1876, *Garber* (Field Mus. Herb.).

Illinois: in a damp meadow near Peoria, coll. of 1903, *McDonald* (Field Mus. Herb.); river bottom opposite Decatur, April, 1864, *Stewart* (Field Mus. Herb.); Eldred, Green Co., 9 May, 1891, *Andrews* (Mo. Bot. Gard. Herb.); opposite St. Louis, July, 1839, and May, 1845, *Engelmann* (Mo. Bot. Gard. Herb. and Kew Herb.); Mississippi Valley, St. Clair Co., colls. of 1874, 1875, and 1879, *Eggert* (Mo. Bot. Gard. Herb.); near Falling Spring, 1 June, 1890, *Glatfelter* (Mo. Bot. Gard. Herb.); East St. Louis, 11 June, 1890, *Hitchcock* (Mo. Bot. Gard. Herb.).

Kentucky: Muhlenberg, 5 June, 1901, *Price* (Mo. Bot. Gard. Herb.); without locality, *Short* (Kew Herb.).

Tennessee: in swamps, Rutherford Co., July, 1892, *Bain* (U. S. Nat. Herb.).

Alabama: Tuscaloosa, April, 1892, *Ward* (U. S. Nat. Herb.); Greensboro, coll. of 1857, *Watson* (Gray Herb.); Auburn, Lee Co., 9 April, 1898, *Earle & Baker* (Field Mus. Herb.).

Mississippi: damp fields, North Carrollton, 21 April, 1899, *Clute 24* (Field Mus. Herb.); without locality, coll. of 1843, *Holton* (Kew Herb.).

South Dakota: Fort Pierre, July, 1853, *Hayden* (Mo. Bot. Gard. Herb.).

Missouri: Courtney, 15 May, 1896, *Bush 701* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); vicinity of St. Louis, coll. of about 1840, *Duerinck* (Mo. Bot. Gard. Herb.); Creve Coeur Lake, 8 May, 1859, *Glatfelter* (Mo. Bot. Gard. Herb.); near St. Louis, *Hus 4007* (Mo. Bot. Gard. Herb.); St. Louis Co., 24 May, 1896, *Shannon 250* (Mo. Bot. Gard. Herb.); St. Louis Co., 20 May, 1879, *Eggert* (Mo. Bot. Gard. Herb.); Jefferson Barracks, 6 May, 1890, *Hitchcock* (Mo. Bot. Gard. Herb.); Jefferson Co., 5 May, 1896, *Eggert* (Mo. Bot. Gard. Herb.); Kimmswick, 20 May, 1860, *Engelmann* (Mo. Bot. Gard. Herb.); Kimmswick, 23 May, 1885, *Wislizenus* (Mo. Bot. Gard. Herb.); Sulphur Springs, 14 Aug., 1910, *Sherff 1062* (Field Mus. Herb.); Osage, 13 May, 1901, *Norton* (Mo. Bot. Gard. Herb.);

Batesville, Butler Co., 21 May, 1908, *Smith 534* (Field Mus. Herb.); St. Louis, coll. of 1832, *Drummond* (Kew Herb.); St. Louis, *Riehl 382* (Kew Herb.).

Arkansas: Fulton, 17 April, 1905, *Bush 2354* (Mo. Bot. Gard. Herb.); Fulton, 24 April, 1914, *Palmer 5381* (Mo. Bot. Gard. Herb.); Arkansas Post, 20 March, 1909, *Kellogg* (Mo. Bot. Gard. Herb.); Little Rock, 22 April, 1909, *McNair* (U. S. Nat. Herb.); Little Rock, June, 1886, *Hasse* (Field Mus. Herb.).

Louisiana: without locality, *Hale* (Gray Herb. and Kew Herb.); Gretna, 28 April, 1899, *Ball 315* (Gray Herb., U. S. Nat. Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); between New Orleans and Balize, May, 1829, *Berlandier 556, 1946* (Gray Herb. and Mo. Bot. Gard. Herb.); Balize, April, 1839, *Lindheimer* (Mo. Bot. Gard. Herb.); Baton Rouge, 22 Jan., 1874, *Joor* (Mo. Bot. Gard. Herb.); Holly Ridge, West Carroll Parish, July, 1910, *Mosely* (Field Mus. Herb.); swampy woods, Natchitoches, 16 April, 1915, *Palmer 7253* (Mo. Bot. Gard. Herb.); New Orleans, *Drummond 176, 626* (Kew Herb.); New Orleans, coll. of 26 March, 1847, *Bromfield* (Kew Herb.).

Texas: low ground, San Augustine, 31 March, 1915, *Palmer 7114* (Mo. Bot. Gard. Herb.).

Forma **robustior**, forma nova.

Stout herb; upper stem-leaves 1.5 to 2 dm. long, 8 to 10 cm. wide; the large lateral obovate leaf-lobes alternating with smaller wedge-shaped divisions of the leaf.

Georgia: ditch banks, near Savannah, 21 March, 1882, *J. D. Smith* (Gray Herb.), TYPE. This plant appears to be a giant form with rather marked foliage.

19. **S. Greggii** Rydb. Bull. Torr. Bot. Club **27**: 170. 1900.

S. tampicanus Gray, Pl. Fendl. 109. 1849 (in Mem. Am. Acad. N. S. **4**), not DC.

S. lobatus Gray, Pl. Wright., part 2, 99. 1853 (in Smithson. Contr. **5**), not Pers.

Annual or biennial, glabrous or with a slight tomentum in the leaf-axils and on the upper side of the leaf along the mid-

rib; stems one to several from a common base, 1.5 to 4 dm. high, striate; leaves lyrate to pinnately divided into cuneate to subrotund divisions; inflorescence a terminal corymbose cyme; heads 5 to 8 mm. high, radiate; involucre campanulate, slightly calyculate; bracts of the involucre about 21, linear-lanceolate, 3 to 5 mm. long, glabrous; ray-flowers 8 to 12; disk-flowers 45 to 60; achenes hispidulous.

Distribution: southern New Mexico, western Texas, and northern Mexico.

Specimens examined:

New Mexico: banks of the Rio Grande near El Paso, *Wright 1413* (Gray Herb.).

Texas: valley of the Rio Grande, below Doñana, Mexican Boundary Survey, *Parry 659* (U. S. Nat. Herb.); El Paso, May, 1881, *Vasey* (U. S. Nat. Herb.); southeastern Texas, Sept., 1879 to Oct., 1880, *Palmer 754* (Gray Herb.).

Chihuahua: valley of Rio Parral, near Santa Rosalia, 21 April, 1847, *Gregg 11,* (Gray Herb.) CO-TYPE; valley near Ortiz, 11 April, 1887, *Pringle* (Field Mus. Herb.).

20. **S. imparipinnatus** Klatt, Natur. Gesell. Halle **15** : 333. 1881; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32** : 19. 1902.

S. lobatus Gray, Syn. Fl. N. Am. 1^2 : 394. 1884, and ed. 2. 1886, in part, not Pers.; Coulter, Contr. U. S. Nat. Herb. **2**: 241. 1892, in part, not Pers.

Annual or biennial, glabrous or slightly floccose-tomentulose in the axils of the leaves; stems slender, 1.5 to 4 dm. high, simple or branched from the base; leaves 2 to 10 cm. long, 1 to 3 cm. broad, lyrate to pinnately divided or the lowermost occasionally undivided; the upper stem-leaves remote, sessile, and pinnately divided into small linear and entire to abruptly cuneate and unequally toothed lateral divisions; inflorescence a terminal few-headed corymbose cyme; heads 6 to 8 mm. high, radiate; involucre campanulate, glabrous, minutely calyculate; bracts of the involucre usually 21, linear-lanceolate, 3 to 5 mm. long, acute; ray-flowers 8 to 12; disk-flowers commonly 50 to 60; achenes hirtellous-puberulent.

Distribution: western Louisiana, Oklahoma, and Texas.

Specimens examined:

Louisiana: without locality, *Leavenworth* (Gray Herb. and Kew Herb.).

Oklahoma: Rock Creek, coll. of 1884, *Tufts* (U. S. Nat. Herb.); between Fort Cobb and Fort Arbuckle, coll. of 1868, *Palmer 462* (U. S. Nat. Herb.); near Indianola, *Pope* (Gray Herb.); Muskogee, May, 1894, *Schenck* (Field Mus. Herb.); near Paul's Valley, Garvin County, 19 April, 1913, *Stevens 108* (Mo. Bot. Gard. Herb.).

Texas: Dallas, 16 April, 1901, *Reverchon 558* (Mo. Bot. Gard. Herb.); in waste ground, Tarrant Co., 5 May, 1912, *Ruth 367* (Mo. Bot. Gard. Herb.); Waco, *Pace 122* (Mo. Bot. Gard. Herb.); Navarro Co., 22 May, 1880, *Joor* (Mo. Bot. Gard. Herb.); wet ground, Houston, May, 1872, *Hall 368* (U. S. Nat. Herb. and Field Mus. Herb.); Harrisburg, 24 April, 1899, *Eggert* (Mo. Bot. Gard. Herb.); Harris Co., 13 and 22 May, 1876, *Joor* (Mo. Bot. Gard. Herb.); vicinity of Huntsville, 6–12 May, 1910, *Dixon 516* (Field Mus. Herb.); Columbia, 6 April, 1899, *Bush 56* (Mo. Bot. Gard. Herb.); Columbia, 31 March, 1902, *Bush 1263* (Mo. Bot. Gard. Herb.); along Corpus Christi Bay, 21 March, 1894, *Heller 1476* (Gray Herb., U. S. Nat. Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); Corpus Christi, 7 April, 1905, *Tracy 8927* (Field Mus. Herb. and Mo. Bot. Gard. Herb.); low prairies near Rosenberg, 5 April, 1900, *Eggert* (Mo. Bot. Gard. Herb.); Richmond, 15 March, 1914, *Palmer 4954* (Mo. Bot. Gard. Herb.); Hungerford, 4 March, 1914, *Palmer 4844* (Mo. Bot. Gard. Herb.); Austin, March, 1870, *Bodin 52* (U. S. Nat. Herb.); "Bejar a la villa de Austin," *Berlandier 1741, 421* (Gray Herb.), CO-TYPE; near Belknap, 20 April, 1858, *Sutton Hays 515* (Field Mus. Herb.); Brazos, coll. of 1889, *Nealley 91, 280* (Field Mus. Herb. and Mo. Bot. Gard. Herb.); Brazos, April, 1859, *Lindheimer* (Mo. Bot. Gard. Herb.); bottom land between Laredo and Palafox, *Schott* (Field Mus. Herb.).

21. **S. Millefolium** Torr. & Gray, Fl. N. Am. 2: 444. 1843; Gray, Syn. Fl. N. Am. 1²: 392. 1884, and ed. 2, 1886; Chap-

man, Fl. Southern U. S., ed. 3, 266. 1897; Small, Fl. Southeastern U. S. 1305. 1903, and ed. 2, 1913.

An herbaceous perennial, glabrous or with a white floccose-tomentum at the base of the stem and in the axils of the leaves; stems 3 to 7 dm. high, striate; leaves bi–tri-pinnately dissected into linear segments; basal and lower stem-leaves petiolate, 1 to 2.5 dm. long, 1.5 to 6 cm. wide, the upper ones sessile; inflorescence terminating the stem in a corymbose cyme; heads 8 to 10 mm. high, radiate; involucre campanulate, sparingly calyculate, glabrous; bracts of the involucre 4 to 6 mm. long; ray-flowers 8 to 12; disk-flowers numerous, usually 50 to 60; achenes hirtellous-puberulent.

Distribution: mountains of North Carolina and South Carolina.

Specimens examined:

North Carolina: slope of Caesar's Head, 3 Sept., 1876, *Engelmann* (Mo. Bot. Gard. Herb.); without locality, coll. of 1888, *Boynton* (U. S. Nat. Herb.); dry, rocky places on White Oak Mountains, Polk Co., alt. 850 m., 4 May, 1897, *Biltmore Herb. 1301*[b] (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.); Skyuka Mountains, Polk Co., 25 May, 1899, *Churchill* (Gray Herb.).

South Carolina: Table Rock, coll. of 1842, *Buckley* (Gray Herb. and Mo. Bot. Gard. Herb.); "Carolina," *Fraser* (Gray Herb.), part of TYPE; Caesar's Head, Aug., 1876, *Canby* (U. S. Nat. Herb.).

22. **S. tampicanus** DC. Prodr. **6**: 427. 1837; Hemsl. Biol. Cent.-Am. Bot. **2**: 248. 1881, excl. plant of Wright.

S. Ervendbergii Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32**: 19. 1902; Field Col. Mus. Bot. Ser. **2**: 275. 1907.

Glabrous throughout; stem 4 dm. or more high, terete, striate, leafy; leaves thin, pinnately divided into cuneate to obovate, unequally dentate divisions; lower leaves petiolate, 1 to 3 dm. long, the upper ones sessile and amplexicaul by a large stipular-like base; inflorescence a terminal compound corymbose many-headed cyme; heads small, 5 to 7 mm. high,

radiate; involucre campanulate, glabrous, minutely calyculate; bracts of the involucre 21, linear-lanceolate, 3 to 4 mm. long; ray-flowers about 13; disk-flowers numerous, 70 to 90; achenes hirtellous along the ribs.

Distribution: eastern Mexico.

Specimens examined:

Tamaulipas: Tampico, coll. of 1827, *Berlandier 186* (Berlin Herb., tracing and fragments in Gray Herb.), CO-TYPE.

Vera Cruz: Wartemberg, near Tantoyuca, coll. of 1858, *Ervendberg 90* (Gray Herb.); without locality, *Liebmann 172* (Copenhagen Herb., tracing and fragments in Gray Herb.).

Puebla: near Metaltoyuca, alt. 240 m., 27 Feb., 1898, *Goldman 74* (U. S. Nat. Herb. and Gray Herb.).

San Luis Potosi: without definite locality, *Parry & Palmer 533* (Gray Herb.).

23. S. hypotrichus Greenm.[1]

S. Sanguisorbae Hemsl. Biol. Cent.-Am. Bot. 2: 246. 1881, in part, not DC.

An herbaceous perennial; stem 7 dm. high, erect, striate, glabrous, somewhat purplish, branched above; leaves pinnately divided into cuneate to rhomboic-ovate dentate unequal divisions, glabrous above, crisp-hirsute beneath; lower leaves including the petiole 2 to 3 dm. long, 4 to 9 cm. broad, the upper stem-leaves sessile, semiamplexicaul and gradually reduced towards the terminal corymbose cyme; heads 8 to 10 mm. high, radiate; involucre campanulate, sparingly calyculate; bracts of the involucre usually 21, linear-lanceolate,

[1] **Senecio hypotrichus** Greenm. sp. nov. herbaceus perennis; caule erecto circiter 7 dm. alto tereti striato stramineo vel plus minusve purpurascenti glabro, superne ramoso; foliis pinnatifidis, inferioribus petiolatis usque ad 3 dm. longis, 4 to 9 cm. latis, superioribus sessilibus et semiamplexicaulibus gradatim reductis, laciniis anguste cuneatis vel obovatis vel rhombo-ovatis subcrenato-dentatis supra glabris subtus crispo-hirsutis; inflorescentiis terminalibus corymboso-cymosis; capitulis 8–10 mm. altis radiatis; involucri squamis plerumque 21 lineari-lanceolatis 5–6 mm. longis glabris; flosculis liguliferis saepius 13, ligulis oblongis, 6–7 mm. longis, 2.5 mm. latis, 4–5-nerviis; floribus disci 60–70; achaeniis glabris. —Region of San Luis Potosi, Mexico, alt. 1830–2440 m., coll. of 1878, *Parry & Palmer 533* (U. S. Nat. Herb.), TYPE. The Gray Herbarium specimen of Parry and Palmer's No. 533 differs from the United States National Herbarium specimen above cited in having glabrous leaves, smaller and more numerously flowered heads and hirtellous achenes; it has been referred to *S. tampicanus* DC.

5 to 6 mm. long, glabrous; ray-flowers 13, rays oblong, 6 to 7 mm. long, 2.5 mm. broad, 4–5-nerved; disk-flowers 60 to 70; achenes glabrous.

Distribution: central Mexico.

San Luis Potosi: "region of San Luis Potosi," alt. 1830–2440 m., coll. of 1878, *Parry & Palmer 533* (U. S. Nat. Herb.), TYPE.

24. **S. Sanguisorbae** DC. Prodr. **6**: 427. 1837; Hemsl. Biol. Cent.-Am. Bot. **2**: 246, 1881, in part; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32**: 19. 1902.

An herbaceous perennial; stem erect, 3 to 10 dm. high, striate, glabrous, simple or branched; leaves pinnately divided, the radical and lower stem-leaves petiolate including the petiole 1 to 4 dm. long, 3 to 13 cm. broad, glabrous on both surfaces or slightly subarachnoid beneath, the upper stem-leaves sessile and more or less amplexicaul; lateral leaf-segments oblong-cuneate to oblong-ovate, 1 to 7 cm. long, .3 to 5.5 cm. broad, rather coarsely dentate, the terminal segment usually broadly obovate; inflorescence a terminal many-headed corymbose cyme; heads 6 to 8 mm. high, radiate; involucre narrowly campanulate, sparingly calyculate; bracts of the involucre 8 to 13, linear-lanceolate 4.5 to 6 mm. long, glabrous; ray-flowers 5 to 8; disk-flowers 15 to 25; achenes glabrous.

Distribution: southern Mexico.

Specimens examined:

Hidalgo: by brooks, Sierra de Pachuca, alt. 3050 m., Aug., 1902, *Pringle 9959* (Gray Herb. and Mo. Bot. Gard. Herb.); Sierra de Pachuca, 1 Sept., 1903, *Rose & Painter 6739* (Gray Herb.).

Mexico: Toluca, coll. of 1854, *Schaffner* (Gray Herb. and Berlin Herb.); Valley of Mexico, Sante Fe, *Bourgeau 832* (Gray Herb., U. S. Nat. Herb., Berlin Herb., and Kew Herb.); without locality, *Gregg 691* (Mo. Bot. Gard. Herb.); Cima, 24 Aug., 1910, *Orcutt 3767* (Mo. Bot. Gard. Herb.); in moist soil along brooks, Mt. Ixtaccihuatl, alt. 3050–3350 m., Nov., 1905, *Purpus 1514* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); in moist soil, Mt. Popocatepetl, Sept., 1908, *Purpus*

3044 (Field Mus. Herb. and Mo. Bot. Gard. Herb.); Mt. Popocatepetl, 7 and 8 Aug., 1901, *Rose & Hay 6069* (U. S. Nat. Herb.); without locality, *Uhde 582, 602, 603, 609, 624* (Berlin Herb.); without locality, coll. of 1848–49, *Gregg 673* (Gray Herb.).

Michoacan: Angangueo, *Hartweg 313* (Berlin Herb.); cool summits of mountains near Patzcuaro, 2 Aug., 1892, *Pringle 4129* (Gray Herb., U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

25. S. pinnatisectus DC. Prodr. 6 : 427. 1837; Hemsl. Biol. Cent.-Am. Bot. 2 : 245. 1881; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. 32 : 19. 1902.

Cineraria pinnata La Llav. & Lex. Nov. Veg. Descr. fasc. 1, 26. 1824.

An herbaceous perennial; stem erect, 4 dm. or more high, striate, glabrous or slightly tomentulose; leaves pinnately divided, the lower petiolate, including the petiole 1 to 3 dm. long, 3 to 8 cm. broad, the upper sessile and amplexicaul, at first white floccose-tomentulose, later glabrate except for the persistent tomentum along both sides of the rhachis; lateral divisions of the leaf narrowly oblong, sharply serrate-dentate, terminal division obovate-cuneate; inflorescence a terminal compound compact corymbose cyme; heads numerous, 6 to 7 mm. high, radiate; involucre calyculate, glabrous; bracts of the involucre usually 13; ray-flowers commonly 6 to 8; disk-flowers 15 to 20; achenes glabrous.

Distribution: southern Mexico.

Specimens examined:

Hildalgo: Real del Monte, *Ehrenberg 386* (Berlin Herb. and Gray Herb.), also *386a*, *386b* (Berlin Herb.); Real del Monte, coll. of 1830, *Graham* (Gray Herb. and Kew Herb.).

Michoacan (?): Angangueo, *Chrismar* (Berlin Herb.); "Cuesta de las papao Angangueo," *Schiede* (Berlin Herb.).

Mexico, without definite locality: *Bates*, *Mackenzie*, and also *Parkinson* (Kew Herb.).

This species is closely related to the preceding, but differs in the narrower lateral leaf-segments, slightly smaller heads,

and persistent floccose tomentum along the rhachis or midrib.

26. S. coahuilensis Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32** : 19. 1902; Field Col. Mus. Bot. Ser. **2** : 275. 1907. Plate 19, fig. 2.

An herbaceous perennial, glabrous or essentially so throughout; stem erect, 3 to 8 dm. high, branched, striate; leaves pinnately divided into obovate to subreniform cuneate-dentate divisions, thickish and firm in texture, glabrous on both surfaces or slightly pubescent on the veins beneath; lower leaves including the petiole 1 to 3 dm. long, 2 to 5 cm. broad, the upper stem-leaves sessile and amplexicaul; inflorescence terminating the stem and branches in a compound corymbose cyme; heads 7 to 10 mm. high, radiate; involucre campanulate, calyculate with a few small bracteoles, glabrous; bracts of the involucre 13 to 18, linear-lanceolate, 4 to 6 mm. long, thickish; ray-flowers 8 to 10, rays oblong, 3 to 5 mm. long, 4-nerved; disk-flowers 35 to 45; achenes ribbed, glabrous.

Distribution: northern Mexico.

Coahuila: Lerios, Feb. to Oct., 1880, *Palmer 755* (Gray Herb., Kew Herb., and U. S. Nat. Herb.), TYPE; without locality, coll. of 1848-49, *Gregg 403* (Gray Herb. and Mo. Bot. Gard. Herb.).

27. S. leonensis Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32** : 19. 1902; Field Col. Mus. Bot. Ser. **2** : 276. 1907. Plate 19, fig. 1.

An herbaceous perennial, more or less lanate-tomentose throughout, somewhat glabrate in age; stem 2 to 3 dm. high, leafy at the base, essentially naked above; leaves petiolate, pinnately divided, including the petiole 8 to 12 cm. long, about 3 cm. broad, at first lanate-tomentulose on both surfaces, later glabrate; divisions of the leaf rather coarsely, somewhat unequally and sharply dentate, the terminal segment subreniform, the lateral ones (3 to 6 on either side) obovate-cuneate; heads few, about 1 cm. high, radiate; involucre campanulate, slightly calyculate and, as well as the bracteate peduncle, tomentulose; bracts of the involucre about 13; disk-flowers numerous, 50 to 60; achenes pubescent.

Distribution: northern Mexico.

Specimen examined:

Nuevo Leon: Sierra Madre, near Monterey, 1 June, 1889, *Pringle 2894* (Gray Herb.), TYPE.

28. **S. montereyana** Wats. Proc. Am. Acad. 25 : 155. 1890; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. 32 : 19. 1902.

An herbaceous perennial, more or less white-tomentose throughout; stems one to several, 2.5 to 4 dm. high, from a rather stout ascending rootstock; leaves mostly radical, including the petiole 1 to 2 dm. long, 1.5 to 3 cm. broad, pinnately divided into narrow, oblong, cuneate to sublinear, entire or few-toothed divisions, at first white-floccose-tomentose on both surfaces, somewhat glabrate above; heads few, 10 to 12 mm. high, radiate, on long naked peduncles; involucre campanulate, calyculate with minute bracteoles, tomentose; bracts of the involucre slightly shorter than the numerous flowers of the disk; ray-flowers about 12; achenes hirtellous-pubescent.

Distribution: northern Mexico.

Specimens examined:

Nuevo Leon: dry shaded ledges of the Sierra Madre, near Monterey, 27 June, 1888, *Pringle 1922* (Gray Herb., U. S. Nat. Herb., Kew Herb., and Mo. Bot. Gard. Herb.), TYPE.

29. **S. zimapanicus** Hemsl. Biol. Cent.-Am. Bot. 2: 248. 1881; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. 32 :19. 1902.

An herbaceous perennial; stems 3 to 4.5 dm. high, simple, leafy below, nearly naked above, striate, more or less pubescent with flaccid hirsute, jointed, and somewhat matted hairs; leaves mostly basal, sessile or essentially so, 3 to 18 cm. long, 1 to 3 cm. broad, pinnately lobed or divided into oblong-ovate dentate divisions, flaccid-hirsute or subarachnoid-pubescent on both surfaces, more densely so beneath; inflorescence a terminal corymbose few-headed cyme; heads large, 10 to 14 mm. high, conspicuously calyculate, radiate; bracts of the involucre commonly 21 (15–21) linear-lanceolate, 7 to 9 mm. long, thickish, glabrous except at the penicillate tip; ray-

flowers 12 to 15, rays oblong, 10 to 12 mm. long; disk-flowers numerous; achenes about 3 mm. long, ribbed, slightly pubescent on the ribs.

Distribution: eastern Mexico.

Specimens examined:

Hildago: Zimapan, *Coulter 423* (Kew Herb.), TYPE.

Tamaulipas: near Miquihuana, alt. 2140 to 2740 m., 10 June, 1898, *Nelson 4492* (Gray Herb. and U. S. Nat. Herb.).

SECT. 5. BOLANDERIANI Greenm.

§ 5. BOLANDERIANI Greenm. Monogr. Senecio, I. Teil, 22, 23. 1901, and in Engl. Bot. Jahrb. 32 : 18, 19. 1902.

Slender, herbaceous perennials; stems erect or nearly so, 1 to 5 dm. high, from a slender more or less horizontal rootstock; leaves undivided and orbicular-ovate to pinnatifid; heads of medium size, about 1 cm. high, radiate; achenes glabrous. Sp. 30–32.

A. Stems 1.5 to 5 dm. high, leafy to the inflorescence.
  a. Leaves usually pubescent beneath; bracts of the involucre 6 to 9 mm. long, more or less hairy.. *30. S. Bolanderi*
  b. Leaves glabrous on both surfaces; bracts of the involucre 5 to 6.5 mm. long, glabrous ..... *31. S. Harfordii*
B. Stems 1 to 2 dm. high, leafy only at the base..... *32. S. Flettii*

30. S. Bolanderi Gray, Proc. Am. Acad. **7** : 362. 1868; Bot. Calif. **1** : 411. 1876, in part; Syn. Fl. N. Am. **1**^{2} : 392. 1884, and ed. 2, 1886, in part; Howell, Fl. N. W. Am. **1** : 379. 1900, in part; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32** : 19. 1902.

A slender herbaceous perennial; stems ascending or erect, from a creeping rootstock, 1.5 to 5 dm. high, striate, often somewhat purplish; radical and lower stem-leaves undivided and crenately lobed-dentate to pinnately divided into oblong, obovate to subrotund, crenate to sharply dentate divisions, glabrous above, usually pubescent beneath, including the petiole .5 to 1.5 dm. long, 1 to 3 cm. broad; the upper stem-leaves sessile; inflorescence terminating the stem in a few-headed subcorymbose cyme; heads 10 to 12 mm. high, radiate; involucre campanulate, calyculate, usually tawny pubescent; bracts of the involucre about 13, linear-lanceolate, 6 to 9 mm.

long; ray-flowers 5 to 8; disk-flowers rather numerous, 25 to 45; achenes glabrous.

Distribution: California and Oregon, near the coast.

Specimens examined:

California: on sand-stone bluffs at the mouth of the river below Mendocino City, May, 1866, *Bolander 4816* (Gray Herb., Field Mus. Herb., and Mo. Bot. Gard. Herb.), TYPE; Humboldt, coll. of 1868–69, *Kellogg & Harford 539* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb); Humboldt, coll. of 1866, *Kellogg 539* (Gray Herb.); Redwoods, Eel River, coll. of 1878, *Rattan 33* (Gray Herb.); near Crescent City, Del Monte Co., June, 1892, *Burt-Davy & Blasdale 1072* (Field Mus. Herb.).

Oregon: Coast Mountains, Lat. 42°, June, 1884, *Howell 162* (Gray Herb.); Newport, June, 1892, *Mulford* (Mo. Bot. Gard. Herb.).

31. S. Harfordii Greenm. Contr. U. S. Nat. Herb. **11**: 597. 1906.

S. Bolanderi var. *oregonensis* Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32**: 19. 1902.

A slender herbaceous perennial, glabrous throughout; stem erect or ascending from a creeping rootstock, 2 to 5 dm. high, usually leafy; leaves mostly pinnately divided into cuneate to subrotund crenate to laciniate-dentate divisions; the radical and lower stem-leaves petiolate, including the petiole 4 to 14 cm. long, 1 to 5 cm. broad, occasionally undivided, subrotund and crenately lobed and the lobes again crenate-dentate, thin in texture, pale green in the dried state; the upper stem-leaves sessile; inflorescence a few-headed corymbose cyme; heads 8 to 10 mm. high, radiate, including the conspicuous yellow rays 1.5 to 2 cm. in diameter; bracts of the involucre usually 13, narrowly lanceolate, 5 to 6 cm. long, acuminate, acute, glabrous; ray-flowers usually 5 (–8); disk-flowers 15 to 25; mature achenes 2.5 to 3.5 mm. long, glabrous.

Distribution: mountains of Washington and Oregon.

Specimens examined:

Washington: on mountains near the Lower Cascades, Skamania Co., 29 May, 1886, *Suksdorf* (Gray Herb.); in

woods, Lower Cascades, 29 May, 1887, *Suksdorf 872* (Mo. Bot. Gard. Herb.); summit of Mt. Adams, 4 Aug., 1899, *Flett 1087* (Piper Herb.).

Oregon: Rooster Rock, June, 1877, *Howell* (Gray Herb.); Cascade Mountains, 31 May, 1868–69, *"Kellogg & Harford,"* namely *Harford & Dunn 540* (Gray Herb.), TYPE; near Bonneville, Multnomah Co., 11 July, 1885, *Suksdorf 572* (Gray Herb.); Multnomah Falls, 25 June, 1904, *Piper 6212* (Gray Herb.); Bonneville, 24 June, 1905, *Palmer* (U. S. Nat. Herb.).

32. **S. Flettii** Wiegand, Bull. Torr. Bot. Club **26**: 137, *pl. 355*. 1899; Greenm. Monogr. Senecio, I. Teil, 23. 1901, and in Engl. Bot. Jahrb. **32**: 19. 1902; Piper, Contr. U. S. Nat. Herb. **11**: 597. 1906.

An herbaceous perennial, 1 to 2 dm. high, glabrous throughout; leaves mostly basal, petiolate, including the petiole 4 to 12 cm. long, 1.5 to 2 cm. broad, undivided, ovate-orbicular and crenate-dentate to pinnately parted, upper stem-leaves few, 1 to 3, incisely pinnate to linear and bractiform; inflorescence terminating the stem in a few-headed corymbose cyme; heads about 1 cm. high, radiate; involucre narrowly campanulate, sparingly calyculate; bracts of the involucre 8 to 13, linear-lanceolate, 5 to 6 mm. long, thickish, glabrous; ray-flowers commonly 5; disk-flowers about 20; achenes glabrous.

Distribution: Washington.

Specimens examined:

Washington: loose rocks, Olympic Mountains, alt. 1830 m., 27 Aug., 1898, *Flett 801* (Piper Herb.), CO-TYPE; Olympic Mountains, Clallam Co., Aug., 1900, *Elmer 2620* (Mo. Bot. Gard. Herb.); Angeles, Clallam Co., 29 June, 1908, *Flett 3351* (U. S. Nat. Herb.); in volcanic sands, Olympic Mountains, alt. 1525 m., Sept., 1890, *Piper 929* (Gray Herb., Mo. Bot. Gard. Herb., and U. S. Nat. Herb.); crevices of volcanic rock, Olympic Mountains, alt. 2135 m., Aug., 1895, *Piper 2196* (U. S. Nat. Herb., Gray Herb., and Piper Herb.); Yakima Region, coll. of 1882, *Brandegee 176* (Mo. Bot. Gard. Herb.).

(To be continued.)

Explanation of Plate

PLATE 17

Senecio mohavensis Gray
California

From the type specimen, Lemmon No. 3129, in the Gray Herbarium of Harvard University.

GREENMAN—MONOGRAPH OF SENECIO

Explanation of Plate

PLATE 18

Senecio durangensis Greenm.
Mexico

From the type specimen, Pringle No. 10105, in the Gray Herbarium of Harvard University.

GREENMAN—MONOGRAPH OF SENECIO

Explanation of Plate

PLATE 19

Fig. 1. *Senecio leonensis* Greenm.
Mexico

From the type specimen, Pringle No. 2894, in the Gray Herbarium of Harvard University.

Fig. 2. *Senecio coahuilensis* Greenm.
Mexico

From the type specimen, Palmer No. 755, in the Gray Herbarium of Harvard University.

GREENMAN—MONOGRAPH OF SENECIO

Explanation of Plate

PLATE 20

Fig. 1. *Senecio Burkei* Greenm.
Canada

From Macoun's No. 69359 in the Gray Herbarium of Harvard University.

Fig. 2. *Senecio saxosus* Klatt
United States

From Baker's No. 770 in the Herbarium of the Missouri Botanical Garden.

GREENMAN—MONOGRAPH OF SENECIO

THE THELEPHORACEAE OF NORTH AMERICA. IV[1]

Exobasidium

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EXOBASIDIUM

Exobasidium Woronin, Naturforsch. Ges. Freiburg Verhandl. **4**: 397–416. *pl. 1–3.* 1867.—Saccardo, Syll. Fung. **6**: 664. 1888.—Hennings, in Engl. & Prantl, Nat. Pflanzenfam. (**I.1****): 103. 1897.

The type species of the genus is *Exobasidium Vaccinii* Fuck. ex Wor.

Fungi parasitic in leaves, shoots, and flowers, which they deform more or less, producing on the surface of these organs an effused hymenium, rarely composed of basidia alone and more usually felt-like and composed chiefly of interwoven hyphae bearing basidia and conidiophores; basidia simple; spores white, simple or septate.

Exobasidium resembles so closely in the thinness of its fructifications such species of *Corticium* and *Peniophora* as *Corticium byssinum, Peniophora asperipilata, P. pilosa,* and *P. subalutacea* that I follow Saccardo and include it with the above genera in the *Thelephoraceae.* Hennings in Engler & Prantl's 'Die Natürlichen Pflanzenfamilien,' has raised *Exobasidium* to ordinal rank but this is not justified by the structure of the many fructifications of *Exobasidium* which I have sectioned; the illustrations in text-books of the structure in section of the fructification are decidedly diagrammatic and simplified.

In his work already cited, Woronin gives a detailed account of the morphology and life history of *Exobasidium Vaccinii* and illustrates this account with three double plates. The interest in this fungus which Woronin's work aroused has

[1] Issued October 8, 1915.

Note.—Explanation in regard to the citation of specimens studied is given in Part I, Ann. Mo. Bot. Gard. **1**: 202, footnote.

resulted in the publication of other species by various authors, whose descriptions contrast sharply with that of Woronin in giving little weight to the morphological characters of the fungus under consideration, but extended description of the form and color of the gall of a particular collection, with passing reference to the occurrence of the fungus upon a hitherto unpublished host. In case of the galls, the descriptions usually fail to state what other forms besides the one mentioned the galls may have on other organs of the new host and likewise omit mention of the different forms they may have at other times in the year than the particular time at which the type collection was made. Woronin's description of *E. Vaccinii* was based upon field observations extended through two seasons, during which more than a thousand specimens were collected. He gives one double page colored plate to show the various types of galls produced by the different organs of *Vaccinium vitis-idaea.*

Plate 21 is a photographic reproduction, reduced one-fifth, of Woronin's colored plate; it shows the forms of galls as determined by the particular organ of the host, *Vaccinium vitis-idaea,* which makes hypertrophic response to local stimulation by the parasitic fungus. A local change of color from green to some shade of red is common in plant portions infested with *Exobasidium.* In the photographic reproduction of Woronin's plate the reddened areas of the original appear light colored. In fig. 1, the left side of the uppermost leaf was attacked by the fungus, producing what I term a leaf spot gall. The affected region of the leaf is reddened on the upper side and bears the fructification which may be felty or scurfy on the under side; this leaf is not distorted much in form and thickness.

Figures 2–9 present leaf galls, reddened on the upper side of the leaf and distorted and thickened by hypertrophic growth so as to become more or less concave with respect to the upper surface. I designate this form of gall as leaf concavity.

Figures 10–17 illustrate shoot galls, in the production of which, stems of the current season's growth have been greatly

enlarged and have turned pale and slightly pink under the stimulus of the infecting fungus. In figs. 10–15 the lateral axillary buds along the infected stem have abnormally enlarged by the stimulation of the fungus and have developed in several instances short, delicate, wax-like or coralloid branchlets of carmine color. Such branchlet shoot galls are beautiful objects in their vegetative condition; they constitute a noteworthy type of gall which is quite different in appearance from the more common leaf galls, produced in response to local infection of leaves. Nevertheless, the common cause of these different gall forms is well brought out by Woronin's illustrations, especially by figs. 11, 12, 13, and 15. Upon shoot galls similar to the above, there have been published *Exobasidium Andromedae* Karst. non Peck for the shoot galls of *Andromeda polifolia, E. cassiopes Peck* for the shoot gall of *Cassiope Mertensiana,* and *E. Oxycocci* Rostrup for that of *Oxycoccus palustris.*

Figures 16–18 show the flower type of gall of *Vaccinium vitis-idaea,* that is, the abnormal growth form made by individual flowers in response to the stimulation of their tissues by the fungus. That both the flower gall and the leaf gall have a common cause has been brought out well by the selection of the specimens used for figs. 16 and 17. In fig. 18 there is presented local infection of a single flower. This is important because isolated flower galls upon a new host have in some cases been regarded as *prima facie* evidence that they have been caused by a new species of *Exobasidium.*

Other host plants produce some types of galls, when infected with *Exobasidium,* which were not figured by Woronin for *Vaccinium vitis-idaea* but which are more or less common. Such gall types are:

(a) Leaf type in which scattered whole leaves of the host are infected. These leaves redden more or less on the upper side and bear on the whole under side the scurfy or felty fructification but are not notably thickened or deformed. This gall differs from the leaf spot gall of Woronin's fig. 1 merely in having the whole of the leaf infected.

(b) Shoot gall with all the leaves toward the tip of the

shoot infected but not deformed. These leaves may be almost normally green on the upper side or they may be more or less reddened, sometimes to carmine red; on the under side they become clothed with the felty fructification of the fungus but the leaves are not deformed. This is merely a more general infection than the leaf type *a*, described above, and is often associated with it on the same plant as well as with the leaf spot and leaf concavity forms.

(c) Bag gall of *Andromeda ligustrina*. This is the extreme in gall production. This gall finally becomes a hollow bag which attains a maximum size of 10–15 cm. in length by 5–10 cm. in diameter. These bag galls are either terminal or lateral on leafy shoots of the current season's growth. When lateral, such a gall has the morphological position of a leaf.

(d) Bud gall of *Symplocos tinctoria*. The expanding leaf buds are deformed into a subglobose mass which may be 3–3½ cm. in diameter. In this gall, the undeveloped stem of the bud is greatly enlarged and the individual leaves of the bud are greatly thickened and deformed.

In North America, we have a large number of species of *Ericaceae* which produce galls when infected by *Exobasidium*. The specimens which have accumulated under *Exobasidium* in herbaria show that none of the gall forms which I have designated under distinctive names in the preceding paragraph are isolated forms. Favorable hosts show a connection and gradation between the various gall forms as intimate as that presented by Woronin for *Vaccinium vitis-idaea*. However, the terms which I employ are useful for contrasting and comparing the data presented by the specimens which I have studied. These data are later given in tabular form.

The microscopic examination of an Exobasidium gall shows that it is composed principally of the tissues of the host plant. Hyphae of the fungus ramify about between the cells of the host and, in the galls in which deformation has taken place, the presence of the fungous hyphae has caused the host both to multiply and enlarge its cells in the infected region. The gall is, therefore, a direct product of the host plant, which

is stimulated to growth by the presence of the parasitic vegetative hyphae, by absorption of organic products from the host, and, undoubtedly, by excreta from the hyphae. We may see from Woronin's figures that the various organs of a given host produce different galls when infected by the same fungus; from which we may conclude that the several organs of the host make different growth responses to the same stimulating cause. We have in the host itself, in its several organs, and also in the age of tissues of these organs, as I shall point out later, factors not only able to produce, but actually producing, diversity in gall form even though but a single species of *Exobasidium* is the parasitic stimulant. Of what value, then, is the form of the gall as a taxonomic character for species of *Exobasidium?*

The different organs of the host differ in the resistance which they offer to infection by *Exobasidium.* Woronin notes in his work cited that out of more than a thousand specimens of *Exobasidium Vaccinii,* only twelve showed flower galls. Hence the flowers of *Vaccinium vitis-idaea* are much less subject to infection than the leaves. In only the one case, which he illustrates by fig. 18, did he observe local infection of a flower. In figs. 16 and 17, the infected flowers are borne on infected shoots and may have become infected through these shoots. We may therefore conclude that in a given host a high resistance of certain organs to infection by *Exobasidium* restricts the galls for that host to fewer organs and to a smaller number of forms than in some other host with a lesser resistance.

That the age of the organs, or their cells, of a host is an important factor in the determination of gall form is apparent if one observes throughout a season the succession of galls produced by a favorable host. In this connection Richards[1] has stated, "and also on Gaylussacia resinosa in the earliest formed distortions, whole shoots are transformed. Later in the season the Exobasidium forms only slight local distortions on the leaves, and still later one finds forms which do not distort the tissues of the host plant at all, but simply form a

[1]Bot. Gaz. 21 : 107. 1896.

scurf on the lower side of the leaves. The same succession is found in the forms on Andromeda down to the last mentioned." Richards determined by culture experiments that the remarkable bag galls of *Andromeda ligustrina* are merely early (June in Massachusetts) productions under the same specific fungous stimulus which later in the season induces leaf concavities on this host. The account of his experiments[1] may be summarized as follows: During July, Exobasidium spores were removed with suitable precautions from fresh mature bag galls of *Andromeda ligustrina* and were immediately transferred to buds and young leaves of experimental plants of the same species, which were isolated in a moist chamber. In about ten days faint discolorations of the leaves were noticed, at first yellowish and then pink. About five days later, the spots which had considerably enlarged, began to show unmistakable signs of thickening, forming the peculiar concavities in the leaves seen in other Exobasidia. In external form, and also in the matter of basidia and spores, this distortion resembled precisely the leaf form on *Andromeda ligustrina,* and indicates that the *Exobasidium* which produces the bag galls of the young buds is identical with the fungus which produces the leaf form found later in the season.

The foregoing presentation of the Exobasidium gall as a growth response of the host under stimulation by the fungus shows that very different forms of galls and differences in regard to abundance of each form on a host may result—

(a) From the different organs making the response.

(b) From differences in resistance of the several organs, which, in many cases, may undoubtedly be so great as to give complete immunity for certain organs.

(c) From the age of the organ attacked.

Since the host produces a great variety of gall forms as growth responses to attack by a single species of *Exobasidium,* how are we to decide whether a given gall form is ever sufficiently distinct to entitle its causative organism to separate specific rank? Gall forms are host products to so large an

[1] *loc. cit.,* p. 105.

extent that they can have little, if any, value for discriminating between species of *Exobasidium*. Into the formation of such galls so many other factors besides the Exobasidium hyphae enter that it is impossible to consider galls as homologous with the fructification of an ascomycete or that of a toadstool, and they should not be used therefore in the way these true fungous fructifications are used for affording in their form specific characters. As a matter of fact, the layer of basidia and conidia-bearing hyphae at the outside of the gall comprise the whole fructification of the parasitic fungus; this layer alone is morphologous with a toadstool. The mere form of the foreign substratum covered by the resupinate fructification of *Exobasidium* should have no greater taxonomic weight than it has in the closely related genus *Corticium*.

We should now consider the distribution of *Exobasidium Vaccinii* as a parasite upon various genera and species of the *Ericaceae*. Woronin limited his investigation of *E. Vaccinii* to what he observed on *Vaccinium vitis-idaea* and left the matter there for other investigators to go on with, if they were so disposed. As the collections which are made on this host nearly always show the fungus occurring in leaf spot galls and leaf concavity galls, and since these forms of galls are the only ones on this host common enough for distribution in published exsiccati, the species *Exobasidium Vaccinii* seems to have become altogether too closely associated with, and limited in mycological practice to, merely the very commonest gall forms which are produced under stimulation by *E. Vaccinii*. For example, Shear[1] states, "The typical form of *Exobasidium Vaccinii* occurs on *Vaccinium vitis-idaea*, producing hypertrophied spots on the leaves. No record has been found of the occurrence of hypertrophied shoots on this host similar to those found on cranberry plants. Rostrup[51] seems to have been the first to describe this form. In 1883 he reported it as occurring on *Oxycoccus palustris* in Denmark."

[1] Cranberry Diseases. U. S. Dept. Agr., Bur. Pl. Ind., Bul. 110: 36. 1907.

Without doubt, this misapprehension of the galls produced by *Vaccinium vitis-idaea* is due to the scarcity of copies of Woronin's original account of *Exobasidium Vaccinii,* for Woronin is at great pains to show that to *E. Vaccinii* are due both shoot galls and flower galls.

That the erroneous tendency of limiting to *E. Vaccinii* the production of only the commonest leaf galls is potent, is apparent from inspection of the table towards the close of this paper where under the heading, "Exobasidium Vaccinii (Fuck.) Wor. The following have been referred here invariably" there are grouped all Exobasidium galls produced by *Vaccinium vitis-idaea, V. vacillans, V. arboreum, V. pennsylvanicum, V. stamineum, Gaylussacia frondosa, G. resinosa, Arctostaphylos uva-ursi, A. nevadensis, Arbutus Menziesii, Rhododendron canadense, R. maximum,* and *Lyonia jamaicensis.*

Our *Gaylussacia frondosa* and *G. resinosa* of this list merit some detailed consideration for they compare very favorably with *Vaccinium vitis-idaea* as hosts for *Exobasidium Vaccinii.* The galls of these two species of *Gaylussacia* include during the season two shoot forms, leaf concavity type, leaf spot type, and the flower type. The flower type of gall is probably very rare; I have seen a dried herbarium specimen of it collected by Dr. Farlow, at Brewster, Massachusetts, and two others, preserved in alcohol in Seymour Herbarium, one of which was collected by A. B. Seymour, at Woods Hole, Massachusetts, and the other by Mrs. Pier, at Biddeford, Maine. These flower galls have a diameter of 10–12 mm.; all the floral organs are enlarged as in case of the flower galls illustrated by Woronin. Bartholomew collected and distributed in his 'Fungi Columbiani,' 3429, the shoot gall of the wax-like or coralloid type such as is produced by *Vaccinium vitis-idaea. Gaylussacia resinosa* very frequently produces as its earliest galls the other form of shoot gall with all the leaves felty on the whole under surface, more or less reddened above, and not deformed. Such a shoot gall is produced by *Vaccinium Myrtillus* in Europe; it has usually been regarded by European mycologists as due to *Exobasidium Vaccinii.* Its regular

occurrence in North America in a series of *E. Vaccinii* forms confirms the correctness of the reference.

As we take up the consideration of North American species of *Exobasidium* which have been published since 1867, we find that in nearly all cases peculiarities of galls have furnished the distinctive portion of the description. These odd or striking forms of galls have been discovered upon new hosts, as was to be expected, for a new host species would without doubt have composition and properties at least slightly different from those of *Vaccinium vitis-idaea*—so different that the growth response, i. e., the gall of this new host, might differ somewhat, perhaps differ notably, from that of *V. vitis-idaea,* even though the stimulus should be given by the same fungus. Two of the specific names to be considered are based entirely upon the occurrence of *Exobasidium* on a new host, and the other eight are founded upon more or less noteworthy galls. Reference to the second division of my table shows that gall form rather than host has caused the publication of specific names in *Exobasidium.*

Exobasidium Peckii, for example, was published as the cause of flower galls produced by *Andromeda Mariana.* Its flower galls are produced so frequently that they attracted attention; leaf concavity galls are common here also. The morphological characters of the fungous cause of these galls agree closely with those of *Exobasidium Vaccinii,* and the galls themselves are of types that *Vaccinium vitis-idaea* produces under stimulation by *Exobasidium Vaccinii.* No evidence of any nature has been offered tending to show that *E. Peckii* is not *E. Vaccinii* in all respects. The frequent production of flower galls by *Andromeda Mariana* can be simply accounted for as due to the susceptibility of the young flower to infection by the fungus, that is, to a special property of this host. I regard *Exobasidium Peckii* as a synonym of *E. Vaccinii.*

In connection with the discussion of *E. Peckii,* attention should be called to occasional flower galls produced by *Lyonia* (*Andromeda*) *ferruginea.* I have seen only four specimens of these galls, two from Georgia and two from Florida. All

resemble monstrous flowers—up to 5 cm. long in the dried state—with all floral organs enlarged proportionally, as in the flower galls of *Andromeda Mariana, Gaylussacia resinosa,* and *Vaccinium vitis-idaea.* Only flower galls are as yet known to me for *Lyonia ferruginea,* but as the morphological characters of the fungus found on the galls are those of *Exobasidium Vaccinii,* I regard these galls as similar to those of *Andromeda Mariana* but much larger and due to *Exobasidium Vaccinii.* The large size of these Lyonia galls is the expression of the growth response of the flower tissue of this host. It will be interesting if further collections of this host show that only the flowers are susceptible to infection by *Exobasidium.*

Exobasidium Oxycocci was proposed as a name for the fungus causing the shoot galls of wax-like or coralloid habit which are produced by *Oxycoccus palustris.* Similar galls are produced in the United States by *Vaccinium macrocarpon* and *V. intermedium.* Shoot galls of *V. macrocarpon* are illustrated in color by Shear[1] and also the leaf spot and leaf concavity galls which this host produces. The morphological characters of the fungus producing the shoot galls on the cranberry species of *Vaccinium* are the same as those of *Exobasidium Vaccinii;* the galls produced by cranberry plants are such as *E. Vaccinii* produces. As there is no evidence of any kind that *E. Vaccinii,* common throughout the same region, does not cause the cranberry galls, the name *E. Oxycocci* seems quite unnecessary.

Exobasidium Cassiopes and *E. Karstenii* have been published as causes of the shoot galls produced by *Cassiope Mertensiana* and *Andromeda polifolia* respectively. These shoot galls are of the wax-like or coralloid type such as *Vaccinium vitis-idaea* produces under stimulation by *Exobasidium Vaccinii.* As the morphological characters of the so-called *E. Cassiopes* and *E. Karstenii* are those of *E. Vaccinii,* and as no evidence has ever been presented that *E. Vaccinii* does not cause the galls referred to, *E. Cassiopes* and *E. Karstenii* should also be regarded as synonyms of *E. Vaccinii.*

[1] *loc. cit., pl. 8.*

Exobasidium Andromedae Peck is based on the bag gall produced by *Andromeda ligustrina.* This gall described in detail on a preceding page, is so very large and remarkable in structure that it did seem that here, if anywhere, must be the anomaly for higher fungi of a fungous cause, specifically different from *Exobasidium Vaccinii,* yet having the same morphological characters. From this point of view, Richards' experiment,[1] already described, of growing on the leaves of *Andromeda ligustrina* a July crop of leaf concavity galls from spores produced by a bag gall which had matured at the beginning of July, was very illuminating. It showed that such a bag gall is noteworthy only because it shows peculiar properties inherent early in the season in shoots and leaves of *Andromeda ligustrina,* that this bag gall belongs in the series with, and is caused by, the same fungus as the leaf concavity galls such as *Exobasidium Vaccinii* produces.

Richards made other experiments tending to show that *E. Vaccinii* produces the bag galls on *Andromeda ligustrina.* He demonstrated that the latter species is not immune to undoubted *Exobasidium Vaccinii,* that it is as susceptible to such spores as to those produced by its own bag galls. In July, spores of *E. Vaccinii* gathered from leaf concavity galls of *Gaylussacia resinosa* were transferred to buds and young leaves of *Andromeda ligustrina.* After about the same lapse of time as when spores from the bag galls were used, there appeared on the Andromeda leaves infected with *Exobasidium Vaccinii* distortions very similar to those produced by spores from the bag galls. As the large bag gall was the only occasion for the name *E. Andromedae* Peck, I agree with Richards that this name is a synomym of *E. Vaccinii.*

In confirmation from the herbarium side of the correctness of the above conclusion, I have a specimen collected in Idaho by Professor Piper, 772, on *Menziesia glabella,* which has a small terminal bag gall such as is produced by *Andromeda ligustrina,* and also a leaf concavity gall.

In the light of what we now know about bag galls the names *Exobasidium Azaleae, E. discoideum,* and *E. Rhododendri*

[1] *loc. cit.*

appear superfluous, for their galls pass through the concavity stage and the morphological characters of the fungi concerned differ in no respect from those of *E. Vaccinii.*

Exobasidium Cassandrae was based on a leaf concavity of *Cassandra calyculata.* The new host was the sole basis for this new name and its author closed his description with the comment, "perhaps this is only a form of E. Vaccinii." Since we now regard *E. Vaccinii* as able to infect many species of the *Ericaceae,* the host alone in this case (with the morphological characters of the fungus agreeing with those of *E. Vaccinii*) does not afford sufficient justification for regarding *E. Cassandrae* as distinct from *E. Vaccinii.*

Exobasidium Arctostaphyli was founded on a leaf spot on *Arctostaphylos pungens.* As in the case of *Exobasidium Cassandrae,* there is no evidence whatever that the fungus concerned is not *E. Vaccinii,* the characters of the fungus and its work being quite those of the latter species.

The usual errors in connection with the preceding series of synonyms which are grouped together in the second division of my table are due, it seems to me, to attaching to a strange gall form—a host product—the same weight which one would give to a toadstool, and to ignoring the true fructifications of the *Exobasidium* concerned. In the taxonomy of the *Hymenomycetes,* species are based upon differences in morphological characters. It is so remarkable an innovation in our taxonomic usage in this group of plants to propose a new species which has precisely the same morphological characters as a well-known and established one that it makes it incumbent upon, and an unusual opportunity for, an author so establishing a species to show conclusively the truth of the paradox that actually good and distinct species of *Hymenomycetes* have the same morphological characters. In all the cases which have been considered, no evidence tending toward such proof has been offered. In the above, I but express the views of many of the best mycologists, who have consistently regarded the above-mentioned Exobasidium names as synonyms of *E. Vaccinii.*

Winter[1] wrote of *Exobasidium Vaccinii* in Europe where there is a similar confusion as to species, "der Pilz erzeugt ausnahmslos Formänderungen der verschiedensten Art an den von ihm bewohnten Pflanzentheilen . . . . . Ich finde zwischen den einzelnen verschiedene Nährpflanzen bewohnenden Formen keine wesentlichen Unterschiede."

The specimens which I have studied show that we have in North America perhaps three species of *Exobasidium,* two of which are rare and are present in herbaria in so few specimens that present conclusions concerning them are somewhat tentative. These species are as follows:

1. *E. Vaccinii* (Fuck.) Wor.

This species is common and wide-spread and is parasitic on many ericaceous host plants. There is as yet no evidence of which I am aware tending to show that so-called physiological races or forms with parasitism limited to a particular host exist in this species. This fungus attacks leaves developing leafy shoots, and flowers of susceptible plants, making its most successful infections when these organs are very young. The vegetative hyphae live in the infected organs between the cells, which are stimulated by the presence and activities of the parasitic hyphae to make a more or less marked hypertrophic growth response, termed a gall. The galls are of varied and sometimes strange form according to the host, the organ, and its age. The distribution of the galls upon the host is dependent upon the susceptibility of its various organs to infection.

In fruiting, the hyphae push through the epidermis to the surface and produce there a resupinate fructification which is amphigenous in the case of galls from tissues so young that they form galls of wax-like or coralloid structure, and hypophyllous on the more common leaf galls. The fructification is variable in thickness, consisting sometimes of scattered clusters of basidia but usually with hyphae present in variable quantity between the basidia so that the fructification may attain a maximum thickness of 60–70 μ, as in the case of col-

[1] In Rabenhorst, Krypt. Flora 1[1]: 322. 1884.

lections on *Vaccinium vitis-idaea*. As shown by Richards,[1] these hyphae bear simple, acicular, conidia about 6–9×1–1½ μ. Conidia are nearly always present in the preparations but have been entered only occasionally in my table. The basidia are generally 4-spored. The basidiospores from herbarium specimens are colorless, simple or with some uniseptate, 10–20×2½–5 μ, but are usually about 12–18×3–3½ μ. They are sometimes a little shorter, or a little longer, or a little thinner, or a little thicker, but are so variable within the extremes stated for different collections on the same host within the same regions or distant regions—as will be seen by reference to my table—that a moderate latitude in spore dimensions seems evident.

2. *E. Vaccinii uliginosi* Boud.

The European specimen of this species distributed from Norway in Briosi and Cavara, 'Funghi Paras.,' 261, has a resupinate, hypophyllous felty fructification, 30–45 μ thick, which is composed almost wholly of large basidia, standing close together and presenting in sections the appearance of a distinct palisade layer. This fructification begins below the epidermis and tears the cells of the latter loose and apart from each other and carries them outward between the basidia. The hymenium is abundantly fruited with basidiospores, borne two to a basidium. The spores are simple, colorless, even, curved towards the base, 18–20×6–7 μ. No conidial hyphae could be found between the basidia in this specimen.

The specimen distributed in Eriksson, 'Fungi Par. Scand.,' 286a, has similar spores 16–20×8 μ. This specimen is in poorer condition and does not show basidia clearly. In some places the fructification is composed of very fine, short-celled hyphae, which are not bearing conidia. Both the above specimens are shoot galls with leaves felty below and reddened above.

Professor Piper, 443, collected on *Vaccinium membranaceum*, at Mt. Ranier, Washington, in August, a shoot gall similar to the European specimens and having a well fruited

[1] *loc. cit.*

Exobasidium with 2-spored basidia and spores 16–20×8 μ. The fungus agrees in all respects with the specimen in Briosi and Cavara, 261. Several other collections on *Vaccinium membranaceum* of buff colored leaf concavity and leaf spot galls appear to bear *Exobasidium Vaccinii*. The very thick spores, borne two to a basidium, distinguish *E. Vaccinii uliginosi* from *E. Vaccinii*.

3. *E. Symploci* Ell. & Mart.

This fungus attacks the developing leaf buds of *Symplocos tinctoria* and deforms them into a lobed mass. In fruiting, the hyphae protrude on the surface of the mass and bear acicular, simple, colorless, slightly curved conidia, ranging from about 7×1 μ upward. The largest spores are 24×2 μ, acicular, curved, and of the same form as those of intermediate size and so on down to attached conidia. I have not found any of the largest spores attached, nor have I found basidia. In the original description the reference to spore characters is "conidia hyaline, cylindric, nearly straight, 15–21×2 μ."

I conclude that basidia have yet to be demonstrated for this fungus.

As I have had an opportunity to examine a large number of Exobasidium specimens, collected in widely separated localities, on many hosts and at various times in the growing season, it has seemed that a concise summary of the data obtained in regard to each specimen might prove useful for comparison purposes to others who study our specimens of this genus in the future. Pains have been taken to give the hosts accurately. I am indebted to Dr. J. M. Greenman for aid in host determinations in several cases.

In the matter of spores the stated dimensions are those of the preparations which were studied. No effort was made to study preparation after preparation from the same collection in order to find spores possibly larger or smaller than those of the first preparation which showed the spores well. The dimensions stated are those obtained by treating all specimens in exactly the same way and give such results as herbarium specimens afford.

TABLE I

COMPARATIVE TABLE OF DATA CONCERNING SPECIMENS OF EXOBASIDIUM EXAMINED

Host	Spore measure	Gall	Date	Locality	Coll. or herb.
EXOBASIDIUM VACCINII (FUCK.) WOR. THE FOLLOWING HAVE BEEN REFERRED HERE INVARIABLY					
Vaccinium vitis-idaea	14–16.8×2.8 μ (Wor.)	Leaf spot, leaf concavity—scurfy or felty below and reddish above—shoot gall, flower gall.	May to Sept.	Russia	Woronin's article
	12–15×3–3½ μ	Leaf spot, leaf concavity—scurfy or felty below and reddish above.	July	Germany	Krieger, Fung. Sax., 62
	12–15×3 μ	Leaf concavity, felty below, red above.	Aug.	Sweden	Romell
	12–15×3–3½ μ	Same as preceding.	Aug.	Sweden	Burt
V. vacillans	12–14×3 μ	Many leaves, felty under, reddish above.	June	Mass.	Sey. & Earle, Ec. Fung., 137a
	12×2½–3 μ	Leaf spot, scurfy below, reddish above.	July	Mass.	Sey. & Earle, Ec. Fung., 137b
	12–15×3 μ	Leaf spot, felty below, red above.	July	Mass.	Sey. & Earle, Ec. Fung., 137c
	12–15×3–3½ μ	Many leaves, felty under, reddish above.	June	Md.	Barth., Fung. Col., 3324
	15×3 μ	Same as preceding.	May	D. C.	Barth., Fung. Col., 1728
	12–18×3–4 μ	Same as preceding.	May	Md.	Barth., Fung. Col., 3231
	Conidia 6–9×1 μ	Same as preceding.	May	Mo.	Mo. B. G. Hb., 4949
V. arboreum	15×3–3½ μ	Leaf spot, scurfy below, reddish above.	April	Ala.	Ala. Biol. Surv.
	12–15×3 μ	Same as preceding.	April	Ala.	Mo. B. G. Hb., 4975
V. pennsylvanicum	12–13×3 μ	Leaf spot, scurfy below, reddish above.		Wis.	Mo. B. G. Hb., 4985
	11–13×3 μ	Leaf spot, felty below, reddish above.	Aug.	Wis.	Mo. B. G. Hb., 44414
	Immature	Leaf spot, scurfy below, reddish above.		N. Bruns.	Mo. B. G. Hb., 44415
	11–13×3 μ	Same as preceding.		Minn.	Mo. B. G. Hb., 44416
V. stamineum	12–15×3 μ	Leaf spot, scurfy below, dark red above.	April	Ala.	Ala. Biol. Surv.
	12–15×3–4½ μ	Same as preceding.	April	Ala.	Mo. B. G. Hb., 4976
	12×3 μ	Same as preceding.	May	Ala.	Mo. B. G. Hb., 4971
	12–15×3–3½ μ	Leaf spot, scurfy below, buff and red above.	June	N. Y.	Mo. B. G. Hb., 4991

V. membranaceum	12–14×3–3½ μ	Leaf concavity, scurfy, yellowish buff.	Sept.	Wash.	Suksdorf, 448
	13–15×3 μ	Same as preceding.	Aug.	Wy.	Mo. B. G. Hb., 44413
	15–19×4–5 μ	Barely a concavity, scurfy, yellowish buff and spots red margined.	Aug.	Wash.	Suksdorf, 504
	12–18×4–5 μ	Same as noted for preceding.	Sept.	Wash.	Suksdorf, 504
	Too immature.	Leaf spot, scurfy below, yellowish.	July	Idaho	Mo. B. G. Hb., 4989
Gaylussacia frondosa	12–15×3–3½ μ	Shoot gall—all later leaves of shoot with whole of each felty below, reddish above.	June	Mass.	Bartholomew, Fung. Col., 3323
	Sterile	Leaf spot, leaf concavity, reddish above.	June	N. Y.	Mo. B. G. Hb., 4953
	12–14×3 μ	Leaf spot, leaf concavity, reddish above.	July	N. Y.	Mo. B. G. Hb., 4957
	Sterile	Leaf concavity, scurfy below, red above.	May	Fla.	Mo. B. G. Hb., 44404
	14×3 μ	Leaf spot, scurfy below, buff colored.	Sept.	Mich.	Waite, 118
	Sterile	Leaf spot, scurfy below, buff or red above.	Sept.	Mass.	Mo. B. G. Hb., 4948
G. resinosa	Immature	Whole leaves, scurfy below, reddish above.	May	Va.	Barth., Fung. Col., 3232
	Conidia 6–9×1–1½ μ	Shoot gall—whole leaves felty below, green or slightly reddened above.	May	Md.	Barth., Fung. Col., 3523
	15×3–3½ μ	Leaf concavity, felty below, red above.	May	Md.	Barth., Fung. Col., 3430
	Conidia	Leaf concavity, shoot gall of the V. vitis-idaea coralloid type.	May	Md.	Barth., Fung. Col., 3429
	Immature	Shoot gall of coralloid type, flower gall.	June	Mass.	Seymour Herb., T54
	Sterile	Leaf concavity, felty below, red above.	July	Mass.	Sey. & Earle, Ec. Fung., 488
	Conidia 6–10×1–1½ μ	Leaf concavity, shoot gall with whole leaves felty under, reddened above.	July	N. Y.	Mo. B. G. Hb., 4781
	Conidia 6–9×1 μ	Shoot gall with whole leaves felty under, reddened above.	.....	Wis.	Mo. B. G. Hb., 4961

TABLE I (Continued)

Host	Spore measure	Gall	Date	Locality	Coll. or herb.
G. resinosa — continued	10–12×2½–3 μ Conidia 6–9×1–1½ μ Conidia 6–8×1 μ	Shoot gall of coralloid type, leaf concavity, flower gall. Shoot gall with whole leaves felty below.	July	Maine	Seymour Herb., T55 Mo. B. G. Hb., 4946
Arctostaphylos uva-ursi	Sterile 12–15×3 μ	Shoot gall with all leaves felty below, reddened above. Shoot gall of the V. vitis-idaea coralloid type.	July Aug.	Wash. Col.	Piper, 434 Barth, Fung. Col., 2729
A. manganita	12×3 μ	Whole leaves felty below, reddened above.	July	Cal.	Seymour Herb.
A. nevadensis	16×4½ μ 12–14×3 μ	Shoot gall of coralloid type. Shoot gall with all leaves felty below, dark red above.	July Aug.	Wash. Wash.	Suksdorf, 840 Piper, 428
Arbutus Menziesii	12–15×2–4 μ	Leaf concavity, felty below, red above.	..	Cal.	Ell. & Ev., N. Am. F., 1586b
Lyonia jamaicensis	15×3½ μ	Leaf concavity to leaf bags, drying reddish brown.	March	Jamaica	Mo. B. G. Hb., 44403
Rhododendron albiflorum	Spores soon 3-septate 15–22×4–6 μ Basidia 4-spored 12–20×4–5 μ and as above	Leaf spots, scurfy below, buff colored. Leaf spots, scurfy below, buff colored.	Sept. Sept.	Wash. Wash.	Suksdorf, 841 Suksdorf, 449
R. canadense	Conidia	Leaf spot, scurfy below, reddish above. Same as preceding.	Sept. Aug.	Newf. Newf.	Mo. B. G. Hb., 42608 Mo. B. G. Hb., 4981
R. maximum	12–15×4 μ	Leaf concavity, red.	July	N. Car.	Mo. B. G. Hb., 4951

EXOBASIDIUM VACCINII (FUCK.) WOR. THE FOLLOWING SYNONYMS ARE BASED ON GALL FORMS AS STATED:

Exobasidium Azaleae Peck, = E. discoideum Ell.					
Azalea nudiflora	15–16×3 μ	Terminal bags, lateral leaf bags.	May	Ala.	Ala. Biol. Surv.
	13–18×3–4½ μ	Leaf bag, scurfy leaf spots, reddish above.	May	Ala.	Mo. B. G. Hb., 4964
	Sterile	Leaf bag suspended by a point.	April	Ala.	Mo. B. G. Hb., 4963
	Sterile	Flowers modified into obconic galls.	June	Mass.	A. B. Seymour Herb.
A. cult. sp.	12–15×3–3½ μ	Flower and leaf bag galls.	June	Mass.	Sey. & Earle, Ec. Fung., 489
A. viscosa	13–18×3–3½ μ	Bag gall, suspended from leaf.	July	N. J.	E. & Ev., N. Am. F., 1718
	Sterile	Bag gall, suspended from leaf.	May	Miss.	Mo. B. G. Hb., 4970
	Conidia	Flower and leaf bag galls.	April	Miss.	Mo. B. G. Hb., 4960
	Sterile	Bag gall, suspended from leaf.	Aug.	Mass.	Mo. B. G. Hb., 44405
	18×3½ μ	Leaf spots, scurfy below, reddened above.	Sept.	Mass.	Mo. B. G. Hb., 44410
Exobasidium Rhododendri Cramer					
Rhododendron ferrugineum	12–15×3–3½ μ	Leaf concavity, bag gall suspended from leaf.	Sept.	Switz.	Rabenhorst, Fung. Eur., 1910
	13–15×3–4 μ	Bag gall, suspended from leaf.	Sept.	Germ.	Magnus, Mo. B. G. Hb.
	14×3 μ	Bag gall, suspended from leaf.	Aug.	Austria	Magnus, Mo. B. G. Hb.
	Sterile	Bag gall, suspended from leaf.	Aug.	Switz.	Kunze, Fung. Sel. Ex. 302
Exobasidium Peckii Halst.					
Andromeda Mariana	12–13×3 μ	Leaf concavity, reddened above; flower gall—flower organs all enlarged.	May	Fla.	A. B. Seymour Herb.
	11–15×3 μ	Same gall forms as the preceding.	May	Fla.	Mo. B. G. Hb., 4966
	12–18×3–4 μ	Same gall forms as the preceding.	June	N. Y.	Sey. & Earle, Ec. Fung., 487
	12–18×3–3½ μ	Leaf concavity, felty below, reddened above.	June	N. J.	Ell. & Ev., Fung. Col., 1210
	Conidia 6–9×1–1½ μ	Leaf concavity, reddened above.	June	Fla.	Mo. B. G. Hb., 4954
Lyonia ferruginea	16–18×4 μ	Flower gall, 2½×2½ cm.—all the organs present and proportionately enlarged.	May	Ga.	U. S. Dept. of Agr.

TABLE I (Continued)

Host	Spore measure	Gall	Date	Locality	Coll. or herb.
Lyonia ferruginea —continued	15×3½–4 μ	Same as above—3–5 cm. long, 1–2½ cm. thick.	June	Ga.	Mo. B. G. Hb., 4955
	12×3½ μ	Flower gall of same type as preceding.	April	Fla.	Mo. B. G. Hb., 4962
		Flower gall of same type as preceding.	.....	Fla.	Mo. B. G. Hb., 44409
Exobasidium Andromedae Peck					
Andromeda ligustrina	15–18×3–3½ μ	Bag gall, terminal on shoot.	June	Mass.	H. L. Jones
	15–18×3–3½ μ	Bag gall, terminal on shoot.	June	Mass.	Rush.
	Conidia	Bag gall, terminal on shoot.	June	Mass.	Duggar, Mo. B. G. Hb.
	17×3½ μ	Leaf bag, terminal bag.	June	N. Y.	Shear, N. Y. Fung., 117
	12–16×3 μ	Bag gall, terminal on shoot.	.....	N. J.	Ellis, N. Am. Fung., 107
	12–15×3½–4 μ	Bag gall in the place of a leaf.	April	Fla.	Mo. B. G. Hb., 44326
Menziesia glabella	10–13×2–2½ μ	Bag gall, terminal on shoot.	Aug.	Idaho	Piper, 772
	10–18×1½–2½ μ	Leaf concavity of E. Vaccinii type.			
Exobasidium Cassandrae Peck					
Cassandra calyculata	12–15×3–4 μ	Leaf concavity, felty below, red above.	.....	N. Y.	Peck, Ellis N. Am. F. 722
	12–15×3–4½ μ	Leaf concavity, scurfy below, red above.	.....	N. Y.	Clinton
	15×3½ μ	Leaf concavity, felty below, red above.	Aug.	Canada	Ell. & Ev. N. Am. F., 2312a
	15×3–4½ μ	Leaf concavity, felty below, red above.	July	Mich.	Trelease, Mo. B. G. Hb.
	12×3 μ	Whole leaf, felty below, red above.			
	Conidia	Shoot gall—all leaves felty under, reddish above.	.....	Newf.	Robinson & von Schrenk, Mo. B. G. Hb.
	14×3½ μ	Leaf spot, felty below, red above.	July	Russia	Fung. Rossiae Ex. 72
Exobasidium Arctostaphyli Harkn.					
Arctostaphylos pungens	12–17×3–5 μ	Leaf spot, scurfy below, red above.	.....	Cal.	Ell. & Ev., N. Am. F. 1586a
	12–18×4½ μ	Leaf spot, scurfy below, red above.	.....	Cal.	Harkness, Mo. B. G. Hb.

Exobasidium Cassiopes Peck					
Cassiope Mertensiana	12–13×3 μ	Shoot gall of the V. vitis-idaea coralloid type.	Aug.	Wash.	Suksdorf, 501
	12–13×3 μ	Shoot gall like the preceding.	Aug.	Wash.	Piper, 771
Exobasidium Oxycocci Rostrup					
Vaccinium macrocarpon	15×3–3½ μ Conidia 6–9×1–1½ μ	Shoot gall of the V. vitis-idaea coralloid type.	.....	Mass.	Minns in U. S. Dept. of Agr. Hb.
	12–15×3 μ	Shoot gall like the preceding.	Sept.	Mass.	Trelease, Mo. B. G. Hb.
	12×3 μ	Leaf spot, leaf concavity, scurfy, red above.	Aug.	Mass.	Ell. & Ev., N. Am. F. 2312b
V. intermedium	12–14×3 μ	Shoot gall of coralloid type.	June	Wash.	Piper, 39
Exobasidium Karstenii Sacc. & Trott. = E. Andromedae Karst. non Peck					
Andromeda polifolia	12×3 μ	Shoot gall, coralloid—all the leaves reddish livid.	July	Finland	Karsten
	12–15×3 μ	Shoot gall like the preceding	July	Finland	Thuem., Myc. Univ. 1110
	Sterile	Shoot gall like the preceding.	June	N. H.	Mo. B. G. Hb., 4778
Exobasidium Vaccinii myrtilli (Fuck.) Juel					
Vaccinium Myrtillus	13–15×3 μ	Shoot gall with all leaves felty below, reddened above.	June	Germany	Krieger, Fung. Sax. 665
	Conidia 6–9×1–1½ μ	Shoot gall like the preceding.	May	Germany	Thuem., Myc. Univ. 115
V. uliginosum	12–15×3–4½ μ	Leaf concavity; shoot gall like above.	July	Germany	Krieger, Fung. Sax., 768
	12–14×3 μ	Shoot gall with all leaves felty below, reddish above.	.....	Finland	Karsten
	10–12×3 μ	Shoot gall like the preceding.	.....	Sweden	Eriksson, Fung. Par., 286b
V. deliciosum	11–12×3 μ Conidia 6–8×1 μ	Shoot gall like the preceding.	Aug.	Wash.	Piper, 842
V. sp.	12–14×3–3½ μ	Shoot gall redder above than preceding.	Sept.	Wash.	Suksdorf, 447

TABLE I (Continued)

Host	Spore measure	Gall	Date	Locality	Coll. or Herb.
		EXOBASIDIUM VACCINII ULIGINOSI BOUD.			
Vaccinium uliginosum	18–20×6–7 μ Basidia 2-spored	Shoot gall with all leaves felty below, red above.	Aug.	Norway	Briosi & Cavara, Fung. Par., 261
V. Myrtillus	15–17×7-8 μ	Shoot gall like the preceding.	.....	Norway	Eriksson, Fung. Par., 286a
V. membranaceum	16–20×8 μ Basidia 2-spored	Shoot gall like the preceding.	Aug.	Wash.	Piper, 443
		EXOBASIDIUM SYMPLOCI ELLIS & MART.			
Symplocos tinctoria	7–14×1½–2 μ, perhaps all are conidia	Leaf bud gall, mass 3×2 cm.	March	Fla.	Ell. & Ev., N. Am. F., 1696
	As above	Same as preceding.	March	Fla.	Mo. B. G. Hb., 4968
	8–24×1½–2 μ, perhaps all are conidia	Same as preceding.	April	Ala.	Mo. B. G. Hb., 4969
	Immature	Same as preceding.	April	Ind.	Rhodes, Mo. B. G. Hb.

Systematic Summary

1. Exobasidium Vaccinii Fuck. ex. Wor. Naturforsch. Ges. Freiburg Verhandl. **4**: 397–416. *pl. 1–3.* 1867. Plate 21.

Fusidium Vaccinii Fuck. Bot. Zeit. **19**: 251. 1861.—*Exobasidium Andromedae* Peck, Buffalo Soc. Nat. Hist. Bul. **1**: 63. 1873; N. Y. State Mus. Rept. **26**: 73. 1874.—*E. Azaleae* Peck, Buffalo Soc. Nat. Hist. Bul. **1**: 63. 1873; N. Y. State Mus. Bul. **26**: 72. 1874.—*E. discoideum* Ellis, Torr. Bot. Club Bul. **5**: 46. 1874.—*E. Rhododendri* Cramer in Rabenh. Fung. Eur. 1910. 1875.—*E. Andromedae* Karst. in De Thuemen, Myc. Univ. 1110. 1878; Finland Natur och Folk Bidrag **37**: 153. 1882.—*E. Karstenii* Sacc. & Trott. in Sacc. Syll. Fung. **21**: 420. 1912.—*E. Cassandrae* Peck, N. Y. State Mus. Bul. **29**: 46. 1874.—*E. Arctostaphyli* Harkn. Calif. Acad. Sci. Bul. **1**: 30. 1884.—*E. Myrtilli* (Thuem.) Karst. Finlands Natur och Folk Bidrag **37**: 152. 1882.—*E. Vaccinii Myrtilli* (Fuck.) Juel, Svensk. Bot. Tids. **6**: 364. 1912.—*E. Oxycocci* Rostr. Bot. Tidsskr. **14**: 243. 1885.—*E. Cassiopes* Peck, N. Y. State Mus. Rept. **45**: 24. 1893.—*E. Peckii* Halst. Torr. Bot. Club Bul. **20**: 437. 1893.

Illustrations: Woronin. *loc. cit.*—Richards, Bot. Gaz. **21**: *pl. 6. f. 1–20.*—Petri, Ann. Myc. **5**: 342–346.—Brefeld, Untersuch. Myk. **8**: *pl. 1. f. 17–22.*—Duggar, Fung. Dis. *f. 215, 216.*—Shear, U. S. Dept. Agr., Bur. Pl. Ind. Bul. 110: *pl. 7. f. A–D.*—Juel, Svensk. Bot. Tids. **6**: 353–372. *f. A–C.*—Engl. & Prantl, Nat. Pflanzenfam. (**I.** 1**): 104. *f. 65.*—Other illustrations in many text-books. References to other illustrations in Sacc. Syll. Fung. **19**: 694.

Fructifications hypophyllous or amphigenous, resupinate, effused, scurfy or felty and compact, grayish, consisting of somewhat scattered clusters of basidia or of basidia and fine, suberect, more or less interwoven and branched hyphae which bear conidia and give to the fructification a maximum thickness ranging up to 60–70 μ; basidia with 4 sterigmata usually; basidiospores colorless, simple or with some 1-septate, 10–20$\times$2½–5 μ, but usually about 12–18$\times$3–3½ μ, becoming 3-septate in germinating; conidia simple, 6–9$\times$1–1½ μ.

Parasitic in leaves, young shoots, and flowers of various ericaceous hosts, and stimulating the infected parts to the production of leaf, shoot, or flower galls which bear the fructifications on their surface. Leaf galls are usually somewhat reddish on the upper side and bear the fructification on the lower side.

From Newfoundland to Florida and westward to California and Washington, also in Jamaica.

I have referred here, with some doubt, the *Exobasidium* causing yellow-buff leaf spot galls on *Rhododendron albiflorum,* collected on mountains in Washington by W. N. Suksdorf. The basidia are 20–30×6 μ, with 4 prominent sterigmata; the basidiospores are mostly 18–21×4½–6 μ, and are nearly all 3-septate. Some of these spores are germinating, hence the septation of the spores may possibly be due to their over maturity when collected, combined with weather conditions at that time favorable to germination. Other collections which show the full series of gall forms on this host are desirable and should give the needed information in regard to septation of the spores.

Specimens examined:

Exsiccati: Ellis, N. Am. Fung., 107, 722; Ell. & Ev., N. Am. Fung., 1586a, 1586b, 1718, 2312a, 2312b; Ell. & Ev., Fung. Col., 220, 1210; Bartholomew, Fung. Col., 1728, 2729, 3231, 3232, 3323, 3324, 3429, 3430, 3523; Seymour & Earle, Econ. Fung., 137a, 137b, 137c, 487, 488, 489; Shear, N. Y. Fung., 117; De Thuemen, Myc. Univ., 115, 210, 1110, 1808; Eriksson, Fung. Par., 286b; Jaczewski, Komarov & Tranzschel, Fung. Rossiae Ex., 72; Kunze, Fung. Sel. Ex., 302; Krieger, Fung. Sax., 62, 665, 768; Rabenhorst, Fung. Eur., 1910; Romell, Fung. Scand., 38.

Austria: On *Rhododendron ferrugineum,* Tyrol, *P. Magnus* (in Mo. Bot. Gard. Herb., 4988).

Germany: On *Vaccinium vitis-idaea,* Königstein, *Krieger,* Krieger, Fung. Sax., 62; Bavaria, *De Thuemen,* Myc. Univ., 910; on *Rhododendron ferrugineum, P. Magnus;* on *Vaccinium Myrtillus,* Leipzig, *G. Winter,* De Thuemen, Myc.

Univ., 115; Königstein, *Krieger,* Fung. Sax., 665; on *V. uliginosum,* Altenberg, *Krieger,* Fung. Sax., 768.

Russia: On *Cassandra calyculata,* Novgorod, Jaczewski, Fung. Rossiae Ex., 72.

Finland: On *Vaccinium uliginosum,* Mustiala, *P. A. Karsten;* on *Andromeda polifolia,* Mustiala, *P. A. Karsten;* and also in De Thuemen, Myc. Univ., 1110.

Sweden: On *Vaccinium vitis-idaea,* Femsjö, *L. Romell;* Upsala, *E. A. Burt;* on *Andromeda polifolia, L. Romell,* Romell, Fung. Scand., 38; on *Vaccinium uliginosum,* Eriksson, Fung. Par. Scand, 286b.

Switzerland: On *Rhododendron ferrugineum,* Luzern, *G. Winter* in Kunze, Fung. Sel. Ex., 302; same host, Maderaner Thal, *Cramer,* Rabenhorst, Fung. Eur., 1910.

Canada: on *Cassandra calyculata,* London, *J. Dearness,* Ell. & Ev., N. Am. Fung., 2312a.

Newfoundland: on *Cassandra calyculata,* Pennie's River, *B. L. Robinson & H. von Schrenk* (in Mo. Bot. Gard. Herb., 4779); on *Rhododendron canadense,* Bluff Head, *A. C. Waghorne, 940* (in Mo. Bot. Gard. Herb., 42608); Virginia Water, *B. L. Robinson & H. von Schrenk* (in Mo. Bot. Gard. Herb., 4981).

New Brunswick: on *Vaccinium pennsylvanicum, Hays, 16* (in Mo. Bot. Gard. Herb., 44415).

Maine: on *Gaylussacia baccata,* Biddeford, *Mrs. A. M. Pier* (in Seymour Herb., T55).

New Hampshire: on *Andromeda polifolia,* Shelburne, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 4778).

Massachusetts: on *Vaccinium vacillans,* Arlington, Magnolia, and Medford, *A. B. Seymour,* Sey. & Earle, Econ. Fung., 137a, 137b, 137c respectively; Plymouth, *E. Bartholomew,* Fung. Col., 3324; Weston, *A. B. Seymour, T56* (in Seymour Herb.); Rafes Chasm, *A. B. Seymour, T58* (in Seymour Herb.); Middlesex Falls, *J. G. Jack* (in Seymour Herb.); on *V. macrocarpon,* Woods Hole, *W. Trelease* (in Mo. Bot. Gard. Herb., 4982); Chatham, *Miss Minns,* and also (in U. S. Dept. Agr. Herb.); Harwich, *B.D.Halsted,* Ell.& Ev.,N.Am. Fung., 2312b; Waverly, *A. B. Seymour, T60* (in Seymour

Herb.); on *V. pennsylvanicum,* Rafes Chasm, *A. B. Seymour T59* (in Seymour Herb.); on *Gaylussacia frondosa,* Woods Hole, *W. Trelease* (in Mo. Bot. Gard. Herb., 4948); Plymouth, *E. Bartholomew,* Fung. Col., 3323; on *G. resinosa,* Manchester, *W. C. Sturgis,* Sey. & Earle, Econ. Fung., 488; Falmouth, *A. B. Seymour, T53* (in Seymour Herb.); Woods Hole, *A. B. Seymour, T54* (in Seymour Herb.); Dartmouth, *W. G. Farlow* (in Seymour Herb.); Brewster, *W. G. Farlow* (in Seymour Herb.); on *Andromeda ligustrina,* Cambridge, *Mr. Rush;* Dedham, *H. L. Jones,* and also *B. M. Duggar* (in Mo. Bot. Gard. Herb., 44411); Woods Hole, *W. Trelease* (in Mo. Bot. Gard. Herb., 44410); Hampden, *A. B. Seymour, T51* (in Seymour Herb.); Granville, *A. B. Seymour* (in Seymour Herb.); on *Rhododendron cult. sp.,* Brookline, *A. B. Seymour,* Sey. & Earle, Econ. Fung., 489; on *R. nudiflorum,* Granville, *A. B. Seymour* (in Seymour Herb.); on *R. viscosum,* Woods Hole, *W. Trelease* (in Mo. Bot. Gard. Herb., 44405, 44408).

New York: on *Vaccinium stamineum,* Ithaca, *W. Trelease* (in Mo. Bot. Gard. Herb., 4991); on *Gaylussacia frondosa,* Eastport, *J. Schrenk* (in Mo. Bot. Gard. Herb., 4953); Eastport, *H. von Schrenk* (in Mo. Bot. Gard. Herb. 4957); on *G. resinosa,* Deer Park, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 4781); on *Andromeda ligustrina,* Alcove, *C. L. Shear,* N. Y. Fung., 117; on *A. Mariana,* Westbury, *F. C. Stewart,* Sey. & Earle, Econ. Fung., 487; on *Cassandra calyculata,* Adirondack Mts., *C. H. Peck,* Ellis, N. Am. Fung., 722; Buffalo, *G. W. Clinton.*

New Jersey: on *Andromeda ligustrina, Ellis,* N. Am. Fung., 107; on *A. Mariana,* Newfield, *Ellis,* Ell. & Ev., Fung. Col., 1210; on *Rhododendron viscosum,* Newfield, *Ellis,* Ell. & Ev., N. Am. Fung., 1718; and (in Mo. Bot. Gard. Herb., 4959).

Maryland: on *Vaccinium vacillans,* Rosecraft, *Bartholomew,* Fung. Col., 3231; on *Gaylussacia resinosa,* Lanham, *E. Bartholomew,* Fung. Col., 3429, 3430; *Bartholomew & Swingle,* Fung. Col., 3523.

District of Columbia: on *Vaccinium vacillans,* Takoma Park, *C. L. Shear,* Fung. Col., 1728.

Virginia: on *Gaylussacia resinosa,* Vienna, *E. Bartholomew,* Fung. Col., 3232.

North Carolina: on *Rhododendron maximum, H. von Schrenk* (in Mo. Bot. Gard. Herb., 4951); on *R. nudiflorum, H. von Schrenk* (in Mo. Bot. Gard. Herb., 4950).

Georgia: on *Lyonia ferruginea,* Brunswick, comm. by U. S. Dept. Agr. Herb.; *W. Trelease* (in Mo. Bot. Gard. Herb., 4955).

Florida: on *Gaylussacia frondosa,* Dunedin, *S. M. Tracy, 6649* (in Mo. Bot. Gard. Herb., 44404); on *Andromeda ligustrina,* St. Leo, *Rev. Jerome* (in Mo. Bot. Gard. Herb., 44326); on *A. Mariana,* White Springs, *H. H. Hume, 88* (in Mo. Bot. Gard. Herb., 4966), and also (in Seymour Herb.); *Chapman* (in Mo. Bot. Gard. Herb., 4954); on *Lyonia ferruginea, Chapman* (in Mo. Bot. Gard. Herb., 44409).

Alabama: on *Vaccinium arboreum,* Auburn, *Ala. Biol. Surv.,* and also (in Mo. Bot. Gard Herb., 4975); on *V. stamineum,* Auburn, *Ala. Biol. Surv.,* and also (in Mo. Bot. Gard. Herb., 4976); Auburn, *F. S. Earle & L. M. Underwood* (in Mo. Bot. Gard. Herb., 4971); on *Rhododendron nudiflorum,* Auburn, *Ala. Biol. Surv.,* and also (in Mo. Bot. Gard. Herb., 4964, 4963).

Mississippi: on *Rhododendron viscosum,* Ocean Springs, *F. S. Earle* (in Mo. Bot. Gard. Herb., 4970); and *S. M. Tracy* (in Mo. Bot. Gard. Herb., 4960).

Michigan: on *Galylussacia frondosa,* Lansing, *M. B. Waite, 118* (in U. S. Dept. Agr. Herb.); on *G. resinosa,* Agricultural College, *G. H. Hicks* (in Seymour Herb.); on *Cassandra calyculata,* Republic, *W. Trelease* (in Mo. Bot. Gard. Herb., 4983); Agricultural College, *G. H. Hicks* (in Seymour Herb.).

Minnesota: on *Vaccinium pennsylvanicum,* Hokal, *L. H. Pammel* (in Mo. Bot. Gard. Herb., 44416).

Wisconsin: on *V. pennsylvanicum,* La Crosse, *L. H. Pammel* (in Mo. Bot. Gard. Herb., 44414); Kirtland, (in Mo. Bot.

Gard. Herb., 4985); on *Gaylussacia resinosa,* Kirkland (in Mo. Bot. Gard. Herb., 4961).

Missouri: on *Vaccinium vacillans,* Crystal City, (in Mo. Bot. Gard. Herb., 4949).

Wyoming: on *V. membranaceum,* Teton Mts., *A. Nelson, E. Nelson, 6525* (in Mo. Bot. Gard. Herb., 44413).

Idaho: on *V. membranaceum,* Forest, Nez Perces Co., *A. A. & E. G. Heller, 3465* (in Mo. Bot. Gard. Herb., 4989); on *Menziesia glabella,* Bitter Root Mt., *C. V. Piper, 772.*

Colorado: on *Arctostaphylous uva ursi,* Glacier Lake, *Bartholomew & Bethel,* Fung. Col., 2729.

Washington: on *Vaccinium deliciosum,* Mt. Rainier, *C. V. Piper, 842;* on *V. membranaceum,* Mt. Paddo, *W. N. Suksdorf, 448;* Chiquash Mts., *W. N. Suksdorf, 504;* on *Vaccinium sp.,* probably *V. membranaceum,* Mt. Paddo, *W. N. Suksdorf, 447;* on *V. intermedium,* Seattle, *C. V. Piper, 39;* on *Arctostaphylos uva ursi,* Orchard Point, *C. V. Piper, 434;* on *A. nevadensis,* Mt. Paddo, *W. N. Suksdorf, 840;* Longwire Springs, *C. V. Piper, 428;* on *Cassiope Mertensiana,* Chiquash Mts., Skamania Co., *W. N. Suksdorf, 501;* Olympic Mts., *C. V. Piper, 771;* on *Rhododendron albiflorum,* Chiquash Mts., Skamania Co., *W. N. Suksdorf, 841;* Mt. Paddo, *W. N. Suksdorf, 449.*

California: on *Arctostaphylos pungens, H. W. Harkness* (in Mo. Bot. Gard. Herb., 4972); and also Ell. & Ev., N. Am. Fung., 1586a; on *A. manganita,* Sisson's, Siskiyou Co., *W. C. Blasdale* (in Seymour Herb.); on *Arbutus Menziesii, H. W. Harkness,* Ell. & Ev., N. Am. Fung., 1586b.

Jamaica: on *Lyonia jamaicensis,* Cinchona, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 44403).

2. **E. Vaccinii uliginosi** Boud. Soc. Bot. Fr. Bul. 41: CCXLIV. 1894.

Illustrations: Juel, Svensk. Bot. Tids. 6: 353–372. *pl. 7. f. 5. text. f. D.*

Fructification hypophyllous, resupinate on the whole lower surface of the leaves, felty, 30–45 μ thick, composed of large basidia arranged side by side in a compact hymenium; basidia

with 2 sterigmata; spores colorless, even, curved towards the base, 16–20 × 7–8 μ.

Parasitic on *Vaccinium membranaceum,* which produces shoot galls with all the later leaves of the gall red on the upper side, felty below, and but slightly, if at all, deformed.

Mt. Rainier, Washington. August.

In the original description of this species, the spore dimensions are stated as 25–32 × 8–12 μ. The European specimens in the exsiccati cited below, which European authors refer here, have spores of the dimensions of the American collection. Shoot galls of the type stated are the only form known to be caused by this species, but other forms may yet be found.

Specimens examined:

Exsiccati: Briosi & Cavara, Fung. Par., 261; Eriksson, Fung. Par. Scand., 286a under the name *Exobasidium Vaccinii.*

Norway: on *Vaccinium Myrtillus,* Eriksson, Fung. Par. Scand., 286a; on *V. uliginosum, G. von. Lagerheim,* Briosi & Cavara, Fung. Par., 261.

Washington: on *Vaccinium membranaceum,* Mt. Rainier, *C. V. Piper, 443.*

3. **E. Symploci** Ell. & Mart. Am. Nat. **18**: 1147. 1884.

Fructification amphigenous, resupinate, effused, consisting of lax, slender, colorless hyphae which bear solitary conidia at the tips of very short, lateral, ascending branches; conidia colorless, even slightly curved, acicular, 7–24×1–2 μ; basidia and basidiospores unknown.

Parasitic on *Symplocos tinctoria* which produces bud galls 3–3½ cm. in diameter, lemon yellow, subglobose and sublobate.

Florida, Alabama, and Indiana. March and April.

In the original description it is stated that the galls are distorted flower buds. In a specimen collected in Indiana, the gall is a partially developed leaf bud.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fung., 1696.

Florida: on *Symplocos tinctoria,* Green Cove Springs, *G. Martin* (in Mo. Bot. Gard. Herb., 4968); and in Ell. & Ev., N. Am. Fung., 1696.

Alabama: on *Symplocos tinctoria,* Auburn, *Ala. Biol. Surv.* (in. Mo. Bot. Gard. Herb., 4969).

Indiana: on *Symplocos tinctoria,* Robertsdale, *A. M. Rhodes* (in Mo. Bot. Gard. Herb., 741178).

SPECIES IMPERFECTLY KNOWN

E. decolorans Harkness, Cal. Acad. Sci. Bul. 1: 31. 1884.

"Receptaculum effused, producing conspicuous yellowish-white, orbicular spots, 1–2 cm. in diameter, not at all distorting the leaf; spores appearing upon the under surface, hyaline, straight, μ 7–8 $\times$ 4–5.

"On living leaves of Rhododendron occidentale. Tamalpais [Cal.]. Autumn. 2887."

The above is the original description. I have seen no specimens referable here nor on the host stated.

EXCLUDED SPECIES

E. mycetophilum Peck ex Burt, Torr. Bot. Club Bul. 28: 285–287. *pl. 23.* 1901.

Tremella mycetophila Peck, N. Y. State Mus., Bul. 28: 53. *pl. 1. f. 4.* 1879.

This curious structure on *Collybia dryophila,* I no longer regard as parasitic but, rather, as a teratological production of *C. dryophila,* induced by protracted wet weather during development of the fructification.

(To be continued.)

Explanation of Plate

PLATE 21.

This plate is a photographic reproduction, ×⅘, of Plate 1 by Woronin[1] of the various galls produced by *Vaccinium vitis-idaea* when parasitized by *Exobasidium Vaccinii*. The original plate is colored and with all figures natural size; red colors of the original have photographed light colored.

Fig. 1. Leaf spot gall, on left side of uppermost leaf; the leaf is reddish on the upper side in the infested area, not deformed, and was felty or scurfy on the lower side.

Figs. 2–9. Leaf concavity galls. More or less deformation of the infected region is present here.

Figs. 10–15. Shoot galls of the wax-like or coralloid type. Extended portions of leafy shoots are infected. Figure 11 shows whole branchlets completely hypertrophied.

Figs. 16–17. Flower galls borne on, and a part of, shoot galls.

Fig. 18. Flower gall. Local infection of a single flower, noted as the only such instance observed.

[1] *loc. cit.*

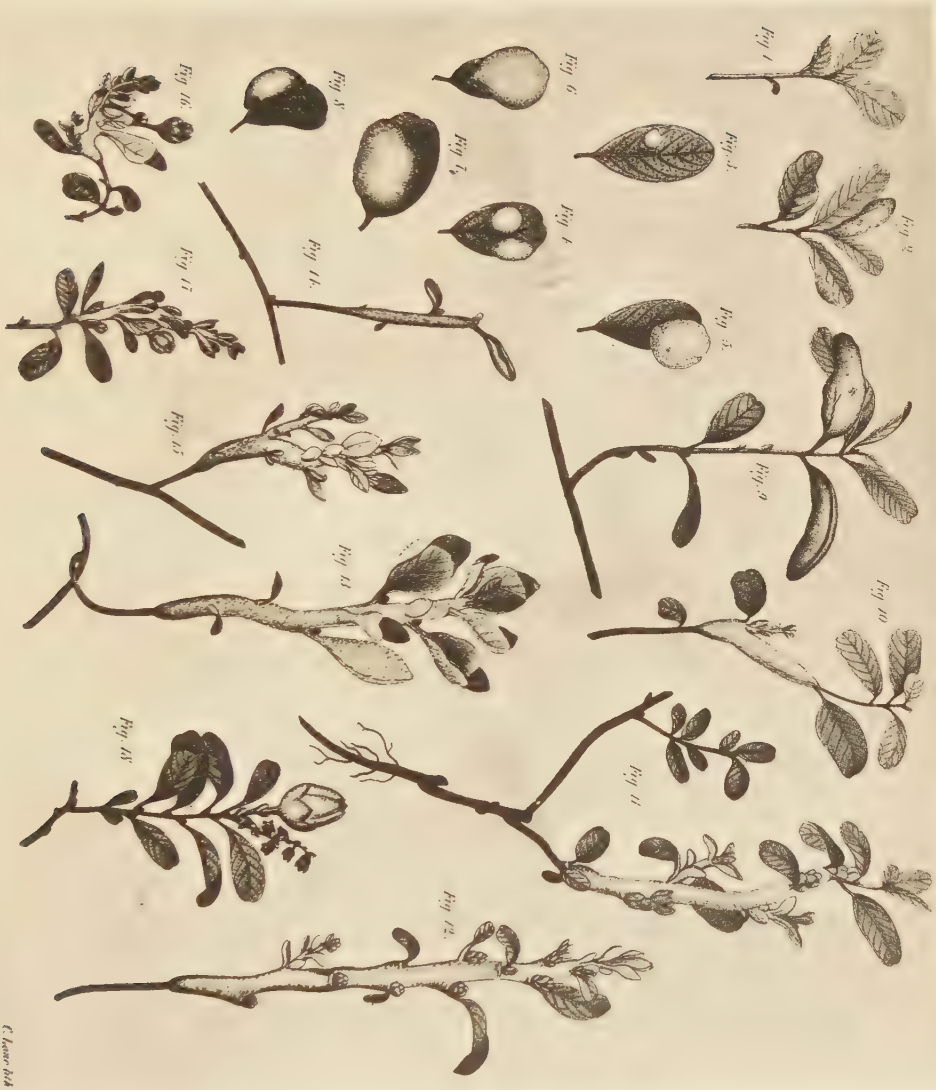
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Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
Fig. 7
Fig. 8
Fig. 9
Fig. 10
Fig. 11
Fig. 12
Fig. 13
Fig. 14
Fig. 15
Fig. 16
Fig. 17
C. Lauer-lith
Tab. I

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TOXICITY OF GALACTOSE FOR CERTAIN OF THE HIGHER PLANTS[1]

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In the course of investigations upon the effect of sugars on the growth of certain higher plants, the sugar galactose was employed. In experiments with vetch (*Vicia villosa*) the plants grown in the presence of 2 per cent galactose showed very marked injury, the injury being especially manifest by a killing of the roots and accompanied by a reduction in the growth of tops. The results secured were the more surprising in view of the fact that lactose sugar employed coincidently influenced beneficially the growth of the same plant. Certain experiments were therefore made to determine whether or not the effect of the galactose was consistent.

Method of experimentation.—The plants were grown under sterile conditions on agar media containing Pfeffer's nutrient solution[2] of one-half its normal strength. This solution is neutral in its reaction. The solution contained varying amounts of galactose sugar, the source of which is indicated in each case.

[1] The writer acknowledges gladly his indebtedness to the officers of the Missouri Botanical Garden for facilities and courtesies extended to him during his stay in St. Louis.

[2] $CaNO_3$	2	grams
KNO_3	0.5	grams
KCl	0.25	grams
K_2HPO_4	0.50	grams
$MgSO_4$	0.50	grams
Fe_2Cl_3	4	milligrams
Dist. water	6	liters

The seed employed were sterilized by means of a method devised in the Laboratory of Plant Physiology of Cornell University by Dr. J. K. Wilson.[1] In brief it is as follows: 10 grams of chloride of lime are shaken up with 150 cc. tap water and after standing for ten minutes the supernatant liquid is filtered. The filtrate is used as the sterilizing agent. The seeds are placed in a test-tube covered with about five times their volume of the filtrate and the tube then tightly stoppered. The seeds are treated for from 4 to 24 hours, depending upon the character of the seed. In the experiments here mentioned the vetch seeds were exposed to this treatment for 12 hours and the peas for 4 hours. The seeds are directly transferred to the culture vessels from the chloride of lime solution, care being observed to drain off all of the chloride of lime solution. In transferring the seed the usual bacteriological precautions are observed.

Experiment with vetch (*Vicia villosa*).—The plants were grown in large glass cylinders 60 cm. high and 10 cm. in diameter, having a volume of approximately 4 liters. In each of the cylinders were placed 250 cc. of the nutrient solution plus 1 per cent washed agar and galactose sugar. The cylinders were then fitted with cotton plugs and sterilized for one hour in an autoclave at a pressure of 15 pounds. The cultures were made in triplicate and the galactose was tested at 2 per cent and at 0.2 per cent concentration. After a growth period of 30 days the cultures showed the injurious action of the galactose, in each case the roots being markedly injured. The primary root tip coming in contact with the agar medium was killed and the lateral root produced met with the same injury, so that ultimately a multi-branched root system was produced after the manner of the pea roots shown in pl. 22 fig. 5. Whatever portions of the roots remained in contact with the agar medium were ultimately killed. It should be mentioned in this connection that the vetch grown in the presence of glucose, saccharose, lactose or maltose at concentrations of 2 per cent was greatly benefited. These sugars are absorbed and assimilated.

[1] Am. Jour. Bot. 2: 420–427. 1915.

Experiment with Canada field pea (Pisum sativum).—In the first experiment with the pea the large cylinders were again employed and to each were added 200 cc. of the nutrient solution plus 1 per cent agar and the sugar whose effect was to be tested. Cultures were made with raffinose, saccharose, lactose, glucose, and galactose ("Merck's Highest Purity"), the concentration of the sugar employed in each case being 2 per cent. The cylinders were fitted with cotton plugs as in the previous experiment and then sterilized for a period of one hour. In each cylinder were sown four peas which had

TABLE I

DATA ON CANADA FIELD PEA

(Duration 25 days. Taken February 13)

Culture	No. of plants	Height of plants cm.	Total green wt. grams	Dry wt. coty-ledons grams	Dry wt. roots grams	Dry wt. tops grams	Total dry wt. grams	Av. dry wt. grams	Gain per plant grams
Glucose	3	44 40 38	6.250	.155	.170	.364	.689	.229	+.085
Lactose	4	40 40 33 33	6.700	.169	.105	.355	.629	.157	+.007
*Raffinose	4	32 33 24 33	6.500	.192	.130	.328	.650	.162	+.012
Saccharose	4	39 35 36 35	7.600	.160	.144	.430	.734	.183	+.036
Check	3	32 23 34	4.450	.150	.075	.190	.415	.138	—.012
Maltose	4	33 40 34 28	6.600	.222	.142	.386	.750	.187	+.034
Galactose	Plants small and roots injured. (See pl. 22, figs. 1a and 5.)								

* Reducing sugar formed in medium probably as a result of secretion of invertase and raffinase from roots. Acidity of entire medium at time of examination equivalent to 0.7 cc. N/10 KOH.

been sterilized by the method described. The plants were grown for a period of twenty-five days and then data taken on the various cultures. The various cultures are shown in pl. 22 fig. 1. The galactose plants are separately shown in pl. 22 fig. 5 and the detailed data are given in table 1.

An examination of the table reveals the fact that every sugar acted beneficially except galactose. If the plants had been examined a month later (as was the case with other cultures), much greater differences would have been secured between the check cultures and the sugar-containing cultures. Lactose is undoubtedly utilized by Canada field pea as well as by vetch and probably before assimilation is converted into glucose and galactose. Raffinose, which is also utilized, yields on hydrolysis first levulose and melibiose, and the latter is further transformed to galactose and dextrose. In the light of the foregoing, it would appear from the results secured with lactose and raffinose that levulose and glucose must exert some protective action against the injurious action of galactose.

Influence of concentration of galactose.—In all of the previous experiments the galactose sugar was employed at only two concentrations, namely, 0.2 per cent and 2 per cent. In the following experiment a series of cultures was made containing galactose at the following concentrations: 0.125 per cent, 0.25 per cent, 0.50 per cent, 1.0 per cent, 2.0 per cent, and control cultures lacking galactose. The plants were grown in large test-tubes 30 cm. × 4 cm., containing 50 cc. of the nutrient medium plus 1 per cent agar. The galactose sugar employed in this experiment was provided by Dr. C. S. Hudson[1], Chief of the Carbohydrate Laboratory, U. S. Bureau of Chemistry. The galactose sugar provided had been recrystallized and was stated by Dr. Hudson to be of a very high degree of purity and probably purer than any which could be secured upon the market. The tubes were plugged with cotton and sterilized in an autoclave at 15 pounds pressure for a period of 20 minutes. One pea was sown in each tube and the cul-

[1] The writer gratefully acknowledges his indebtedness to Dr. Hudson for the galactose furnished.

tures made in triplicate. The seeds germinated in four days and even by this time in the higher concentrations of galactose, browning of the cotyledons was becoming evident. This browning of the cotyledons intensified with time and at the end of 20 days the peas in the 1 per cent and 2 per cent galactose cultures showed marked discoloration, and death of roots soon occurred. The height of tops was also markedly affected in the presence of galactose of a concentration of 1 per cent or over. (See pl. 22 fig. 4.) The above experiments were repeated with wheat and corn and the results secured were similar.

Antagonistic action of glucose toward toxicity of galactose. —It was noted previously that raffinose and lactose are utilized by Canada field pea, and this has been verified by other experiments. The use of lactose by vetch has also been decidedly shown by experiments not yet reported. Since both lactose and raffinose are assimilated by pea and vetch, and since it is highly probable, as previously suggested, that these sugars are hydrolyzed before assimilation, it is possible that the glucose and levulose exercise a protective action against the galactose.

An experiment was made to test the hypothesis with respect to glucose. Test-tube cultures were prepared as in the previous experiment, but in this case were made in quadruplicate. One series contained 1 per cent glucose plus 1 per cent galactose and the second series contained 1 per cent galactose alone. The plants were grown for 25 days in the greenhouse and the general results are clearly evident in pl. 22 figs. 2 and 3. In the case of the 1 per cent galactose culture the primary roots were killed, but with the 1 per cent glucose added, the primary root tip was killed and the epidermis and part of the cortex, but the inner part of the root was not apparently injured, for secondary roots developed which seemed to be more resistant to the toxic action of the galactose, for these root tips suffered no injury and not even a browning of the root was secured as was the case with the primary root (pl. 22 fig. 3). The experiment was repeated a second time and the results secured are concordant with the first.

Discussion.—So far as the writer has been able to discover, no previous mention has been made of the toxic nature of galactose for plants. Molliard[1], however, intimates that galactose is toxic for radish, for unlike other sugars, the galactose permitted no development beyond a 5 per cent concentration and with 2 per cent galactose the plants are very small. He concludes that galactose is not utilized by radish.

That galactose is injurious to the green plants employed is definitely shown. It does not appear to be toxic to fungi since *Aspergillus niger,* several species of *Penicillium,* a species of *Fusarium,* and a species of *Mucor* were all found growing in cultures which became contaminated. It is definitely known also that certain yeasts are able to ferment galactose. The character of the injury effected by the galactose in the above experiment and the method of action have not yet been determined. Incidental observations indicate that the galactose on penetrating kills the cells in its path. In the case of peas grown on 1 per cent galactose the peripheral layers of the cotyledon showed the original starch reserve undigested. In the presence of glucose it was only the epidermis and part of the cortex which suffered injury. It would appear that the outer layers of cells were injured before sufficient glucose had accumulated to render them resistant to the toxic action of galactose, or perhaps the penetrability of the inner cells for galactose was altered by the presence of glucose. In what manner the glucose antidotes the toxicity of galactose cannot yet be stated. It may be possible that it is the oxidation products of galactose that are the injurious agents and that the glucose prevents the formation, or modifies the character, of the oxidation products and that the toxicity is thereby overcome. Investigation into other phases is in progress.

[1] Molliard, Marin. Action morphogénique de quelques substances organiques sur les végétaux supérieurs. Rev. Gén. de Bot. 19: p. 331. 1907.

Explanation of Plate

PLATE 22

Fig. 1. *a*, galactose 2 per cent; *b*, lactose 2 per cent; *c*, check—no sugar; *d*, raffinose 2 per cent; *e*, saccharose 2 per cent; *f*, glucose 2 per cent. (Cotton plugs were removed at the time of photographing.)

Fig. 2. The two outside tubes contain 1 per cent galactose while the two middle ones contain 1 per cent galactose plus 1 per cent glucose.

Fig. 3. The small plant was grown on 1 per cent galactose; the larger one on 1 per cent galactose plus 1 per cent glucose.

Fig. 4. *a* and *b*, 2 per cent galactose; *c*, 1 per cent galactose; *d*, 0.500 per cent galactose; *e*, 0.250 per cent galactose; *f*, 0.125 per cent galactose; *g*, check—no galactose.

Fig. 5. Peas (shown in fig. 1*a*), showing character of root growth when tips alone come in contact with 2 per cent galactose-containing medium.

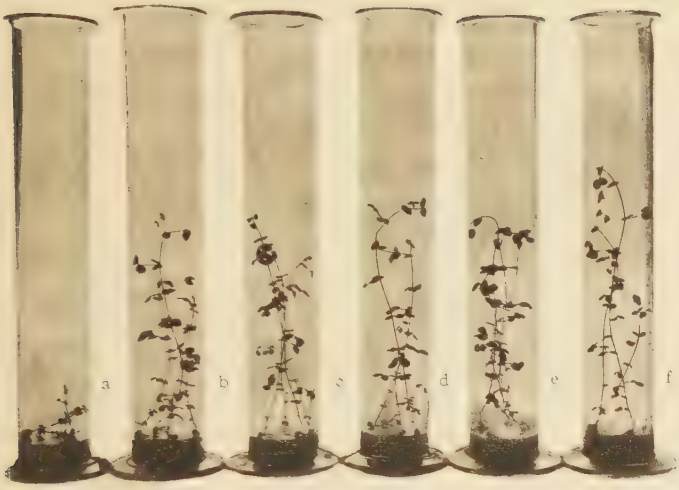

Fig. 1

Fig. 2

Fig. 3

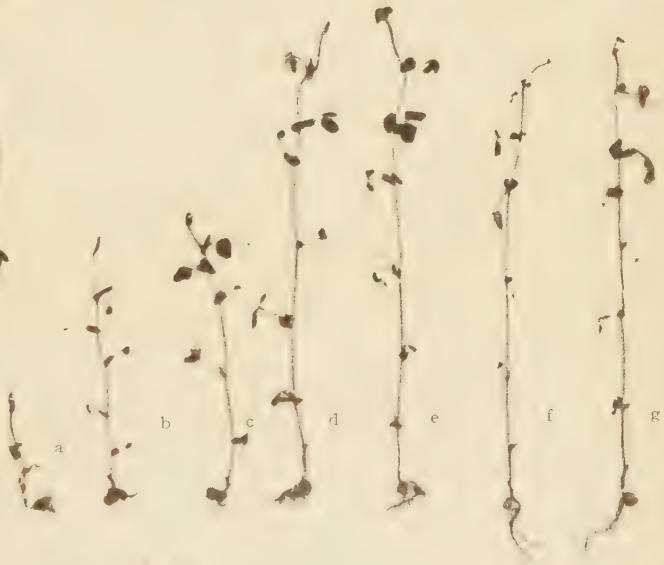

Fig. 4

Fig. 5

COMPARATIVE STUDIES IN THE POLYPORACEAE

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The subclass *Basidiomycetes* of the class *Fungi* contains a natural group of plants sharply separated from related groups in that the hymenium (basidia, paraphyses, etc.) forms the lining of hollow tubes on the ventral surface of the fruit body. This group of plants constitutes the tribe *Polyporeae*. It is divided into two families, the *Boletaceae* and the *Polyporaceae*. The *Boletaceae* are separated from the *Polyporaceae* in that they are fleshy and soon decay and the tubes are easily separated from the pileus, while the *Polyporaceae* vary in texture from coriaceous to hard and woody, and the tubes are inseparable from the pileus. These characters are susceptible of some variation, as there are a very few fleshy species in the latter family, and in two or three cases the hymenium is waxy and the tubes separable. In this article we are concerned only with the *Polyporaceae*.

Historical

Accurate knowledge of the classification of the *Polyporeae* dates back only to the last few years of the eighteenth or the beginning of the nineteenth century. The first attempt worthy of consideration was that of Persoon in 1801, although we still have occasion to refer to articles by earlier writers, especially Bulliard (Herbier de la France, 1780–1793), Schaeffer (Fung. Bav. 1780), and Sowerby (Eng. Fung. 1797–1809). These three, while contributing considerable in the way of illustrations of the species known at that time, knew very little about the correct classification of the species they illustrated. The binomial method of naming species had come into general use following its introduction by Linnaeus (Species Plantarum) in 1753, and many new species were described in the succeeding years, but the descriptions were inadequate and

the type specimens not preserved, so that it is impossible to tell to what plants the descriptions refer.

By the beginning of the nineteenth century those interested in this line of study had begun to feel the need of permanent herbaria containing specimens of all the species described. The appreciation of this need augmented the demand for a more systematic and a more natural arrangement of the genera and species of fungi.

It thus came about that while Linnaeus in 1753 had listed but one genus, *Boletus,* and 12 species of pore fungi (*Boletaceae* and *Polyporaceae*), the number of genera had increased to 3 and the number of species to 93 when Persoon published his 'Synopsis Fungorum,' in 1801. This was followed by the work of Albertini and Schweinitz (Conspectus Fungorum) in 1805, which was modeled after the work of Persoon and contributes nothing to the systematic arrangement of the *Polyporeae.* It must not be supposed, however, that there was any extraordinary change from the incomplete descriptions of the earlier writers to a more or less perfect standard of description that should include all the facts necessary for the identification of the species. The descriptions in Persoon's 'Synopsis' were still far from what could be desired, and it is only where these are supplemented by herbarium specimens or by accurate illustrations or by both that the species can be identified beyond all doubt. But the fact remains that the beginning of the nineteenth century witnessed a growing inclination on the part of mycological systematists toward a form of record for the species that would be more concrete in its conception and thus give an added impetus to the study of the fungi.

Among the vast array of mycologists produced in the nineteenth century by far the most prominent was Elias Fries. His first work of importance was the 'Systema Mycologicum,' published in 1821–1832, in which the known fungi were marshalled in order. To the genera of the *Polyporeae* listed by Persoon he added the genus *Polyporus* (first proposed by Micheli in the eighteenth century) and thus made the first attempt to separate the *Boletaceae* from the *Polyporaceae.*

The genera treated by him contained 164 species in all, of which probably two-thirds were in the single genus *Polyporus*. This genus was divided into 3 sections, *Favolus, Microporus,* and *Polystictus,* the first named being later raised to generic rank. The section *Microporus* contained by far the largest number of species. It was divided into 5 subgenera: *Mesopus, Pleuropus, Merisma, Apus,* and *Resupinatus*. This arrangement was continued in his 'Epicrisis Systema Mycologicum,' published in 1836–38. In the meanwhile the genera *Trametes, Cyclomyces, Hexagona, Favolus, Laschia,* and *Porothelium* had been carved from the old genus *Polyporus,* and the number of species described had increased to 361 (entirely exclusive of the genus *Boletus*). Of these, 280 were included in the genus *Polyporus*. The same disposition of the pore fungi was followed by Fries in his last publication, 'Hymenomycetes Europaei,' in 1874, and, indeed, that system has either been followed in its entirety since or has served as a foundation for all other systems of classification that have been proposed from time to time by others.

Correlated with the increase in the number of described species there is manifest a tendency on the part of some later writers toward a change in the conception of what should constitute a genus. There has been a tendency away from the old idea of large genera containing a heterogeneous collection of species, and toward the breaking up of genera into smaller units consisting of closely related individuals. This tendency finds its best expression in the work of Karsten, Quelet, and Murrill, each of whom has published papers dealing with the classification of the *Polyporaceae*.

IMPORTANT MICROSCOPIC CHARACTERS USED BY EARLIER WORKERS

Having glanced at the beginnings of the various classifications that have been proposed, we may now turn our attention to an analysis of the characters used in separating genera and species. For the most part the generic characters were macroscopic ones, such as presence or absence of a stipe, consistency of the sporophore, nature of the hymenium, etc.,—characters that arrested the attention of the collector without

recourse to the microscope, for the microscope was unknown when the foundations of this study were laid. In the separation of species other macroscopic characters of minor importance were used. Color, pubescence, habitat, form, size, etc., were characters that were largely drawn upon in fixing the limits of species.

It was unfortunate, however, that though the characters named are the most conspicuous ones, yet they are more subject to modification and variation than are certain internal characters that require the use of the microscope for their detection. Perhaps the desideratum in systematic botany would be a classification in which genera are well defined and sharply separated from each other by gross morphological characters, and in which the microscope would be necessary only in determining specific characters. Perhaps this demand is more nearly filled in the family *Agaricaceae* than in any other group of the fungi. There the genera are divided into sections on the color of the spores, and the genera in these sections are more or less well differentiated on gross morphological characters.

In those groups of the fungi that have been most carefully studied, e. g., the *Myxomycetes*, considerable attention has been paid to the minute anatomical structure of the plant. Spore markings that are scarcely visible, except with an oil-immersion lens, have been used as points of separation in closely related forms, and in certain of the *Discomycetes* the spore markings and the nature of the paraphyses have been largely drawn upon to furnish specific characters. Durand[1] has gone somewhat farther, and in his studies in the fleshy *Pezizineae* has taken into account the structure of the apothecium in fixing the limits of the families. Burt[2] has recently set new limits to some of the genera of the *Thelephoraceae*, in keeping with their inner anatomical structure. In the *Polyporaceae*, Miss Ames[3] has recently attempted to outline a scheme of classification of the genera based largely on the structure of the sporophores, but only a few forms

[1] Bul. Tor. Bot. Club 27: 463–495. 1900.
[2] Ann. Mo. Bot. Gard. 1: 195–196. 1914.
[3] Ann. Myc. 11: 211–253. 1913.

were investigated and the results not as satisfactory as could be desired.

It is a significant fact, however, that no attempt has been made to classify the *Polyporaceae* on the basis of spore or other hymenial characters, although it is recognized that, outside of the algae, the organs concerned in reproduction are usually subject to less variation than are external morphological characters. That no such attempt has been made is due to two causes: first, the dislike on the part of students of the careful and painstaking observations that must often be made to determine those characters; and second, to the widespread belief that the pore fungi are spore-bearing only for a short interval of time during the year, and that they must be examined at the right moment or the spores will have disappeared. When it has been shown that the second objection is invalid and that hymenial characters are usually not hard to make out, the first objection will largely disappear.

In the course of the last year the writer has spent a considerable portion of his time in searching for these characters, not only in the *Polyporaceae* but in other related families as well. The methods employed are given on a following page, and suffice it to say here that probably 75 per cent of the collections examined contained spores, and a large percentage afforded other microscopic characters that played a considerable part in distinguishing one species from another. The characters that may be obtained by the use of the microscope are here enumerated and some indication given as to their possible value.

Discussion of Microscopic Characters Now Available for Use as Generic and Specific Characters

The characters that may be obtained by the methods outlined on a following page are as follows: spore characters, presence or absence of cystidia, setae and other sterile organs in the hymenium, basidial characters, hyphal characters, and the presence or absence of sterile structures in the subhymenial tissue.

Spore characters.—Spore characters are probably worthy of a great deal more consideration than they have yet received in the greater part of the mycological work that has been done up to the present time. As previously stated, in the *Agaricaceae* the primary divisions of the family are made on the basis of spore colors. This distinction was made as early as 1821 by Fries in his 'Systema Mycologicum.' The fact that this character was so early recognized was not because spores are more abundant or their colors more striking in the gill fungi, but because the period of spore production more closely coincides with the period of maximum development of the plants. Unfavorable conditions, i. e., drought, superabundance of moisture, cold, etc., result in the disorganization of the tissue in a fleshy fungus, and consequently the duration of the period of spore liberation is permanently shortened. In the coriaceous or woody forms these same conditions result only in a temporary suspension of the act of spore liberation and with the return of normal conditions the suspended function again becomes active. In this way the period during which spores are present in the hymenium of a pore fungus is greatly lengthened, and it is safe to assume that the number of mature spores present at a given time in the hymenium of one of the more durable pore fungi is less than the number of mature spores on an equal hymenial surface of a gill fungus. Contrary to the condition in the *Agaricaceae,* the introduction of spore colors as generic characteristics would mean an entire revision of all the genera, and it may well be doubted whether the advantage obtained from such a limitation of genera would compensate for the confusion that would be sure to arise. On this basis, however, the species could easily be grouped into sections under the genera, but even were that done the white-spored species so far outnumber those with colored spores that the adoption of the idea would delimit only a small group of species that perhaps could be better separated in other ways.

Very little exact evidence bearing on the variation in size in the spores of a given species is obtainable. The work of

Falck[1] showed that the mature spores of certain species of *Lenzites* were very constant in the length of their short axes, the variations being only a fraction of one micron, while the length of the long axis varied considerably, although in that case the variation rarely went beyond 3 μ in different spores from different fruit bodies. Cotton[2] investigated variations in the spores of *Stropharia semiglobata* and found that when the pileus was cut from the stem and a series of spore prints obtained from the former, the spores shed during the first hour measured 18×10 μ, while those shed during the twenty-third hour measured 15×9 μ, and those shed during the eighty-third hour measured only 12×7 μ. The diminution in size was ascribed to the artificial conditions, i. e., the pileus being severed from the stipe, under which the spores were produced. Experiments carried on with sporophores collected and placed in large test-tubes and supplied with water, showed that the spores shed the first day did not differ in size from those shed during the fifth or sixth day. The first experiment suggests the possibility that in plants growing in nature the size of the spores might be reduced if the fungus was growing on a substratum in which the required amount of food substances was not present. No comparative studies along this line have yet been reported and the question of the amount of variation in size of spores is still an open one. However, spore measurements have been very successfully used in separating species of fungi and no doubt the limit of their usefulness has not yet been reached in systematic mycology.

Inaccurate spore measurements may creep into the literature through a misdetermination of species quite as easily as species may be misdetermined because of inaccurate spore measurements. The former condition is especially liable to be pronounced in the literature of a fungous flora as little known as is that of this country, and where species are not determined on microscopic characters, but these same characters are entered in the literature when the species is re-

[1] Moeller's Hausschwamm-forschungen, Heft 3, pp. 79–96. 1909.
[2] Trans. Brit. Myc. Soc. 4: 298–300. 1914.

corded. This latter procedure is entirely commendable, but it has been so much abused that the spore characters carried in the literature are far from being reliable in a large number of cases. However, allowances must be made for some variation in measurement by different individuals as no two persons will report exactly the same measurements for one species.

The shape of the spores is probably subject to somewhat less variation with age than is the size. Spores begin to take their characteristic shape while they are yet comparatively immature and from seeing such a spore one can judge of its mature form more accurately than of its mature size. Often the spores of two or more species are so similar in shape that it is perhaps best not to try to distinguish between them, although the distinction may be perfectly apparent to one who has before him the spores of all the species in question. The terms used to describe spore forms are not as rigidly defined as we could wish, and it does not add to the clearness of distinction between two species to describe the spores of one as "elongate-ellipsoid" and of the other as "narrowly fusoid" and expect the users of the manual to distinguish the species on that basis. There are many cases, however, where the form of the spores may be used to good advantage.

Spore markings are so universally absent in the *Polyporaceae* that the subject requires very little comment here. There are probably not more than a dozen species that are characterized in this way and they are so widely separated that the character is given an added value. In some groups of the fungi, especially among the *Ascomycetes*, not only the presence or absence of markings on the spore wall but also the nature of these markings is taken into account.

Cystidia.—Cystidia may be defined as more or less conspicuous sterile organs found either in the hymenium or in the subhymenial tissue of various basidiomycetous fungi. They are usually unicellular and they may be smooth or they may have a more or less incrusted surface, the incrusting substance probably always being calcium oxalate. The name "setae" has been given to these bodies when they are colored

(usually brown) and sharp-pointed, and that distinction is maintained in this paper, although there may be some doubt as to the advantage that accrues from its use. The presence or absence of setae has been made a generic character in some groups of the *Basidiomycetes,* and even in the *Polyporaceae* the genus *Mucronoporus* was founded by Ellis and Everhart on the presence of the setae in the hymenium. The genus probably has not received the acceptance that it has deserved at the hands of mycologists. It is difficult to say at times whether a given structure should be designated as a cystidium or not, but the writer is of the opinion that the term should be used in its broadest sense, except that it should not be applied to those structures usually referred to as paraphyses. These latter can usually be distinguished by the frequency of their occurrence as they usually alternate with the basidia, while cystidia or setae are scattered irregularly through the hymenium. In by far the largest number of cases the cystidia are very conspicuous on account of their size, coloration, incrustation, or other characters. In a few cases the presence or absence of setae is a variable character, in some specimens being abundant and in others very scarce. In such cases the writer has found it advisable to make longitudinal sections of the tubes, as the setae are sometimes more abundant in one part of the tubes than in another. A cross-section of the tubes of *Fomes igniarius* will sometimes fail to show a single seta, but in only one specimen has the writer failed to locate them in longitudinal sections from the hymenium of the same plant. They are also almost entirely lacking in some specimens of *Polyporus dryophilus.*

Basidia.—It is very seldom that the basidia offer characters that can be used in separating species. They are almost universally 4-spored in the *Polyporaceae* and in those few species where 2- and 3-spored basidia do occur there are always a goodly number of 4-spored ones present also. In a very few cases the basidia are conspicuous on account of their large size. This is true of *Trametes Peckii* where they are 8–10 μ broad, while usually they vary from 3 to 6 μ broad.

Hyphal characters.—The characters of the hyphae that make up the subhymenial tissue and the tissue of the trama of the pileus have never been used in the classification of the *Polyporaceae.* While the size of the hyphae may depend to a considerable degree on the food supply of the plant, yet in examining a large number of species the writer has found that some are characterized by hyphae two to three times as large as in most species. These cases have been thoroughly investigated as far as herbarium material would permit and as all specimens have showed the character about equally well, it has been taken as a means of identifying the species in which it has occurred. The writer knows of no factor or combination of factors that would be operative on a large number of individuals from widely separated localities and in the case of but a limited number of species. If it be dependent on nutrition, then the species possessing this character are so constantly associated with that kind of nutrition that the character is as constant a one as can be obtained. The same is true of the unbranched hyphae of the context of *Polyporus albellus.*

Incrustation of the hyphae has never been observed in the pileate *Polyporaceae,* though it is a well-marked character in the species of certain groups of resupinate fungi.

METHODS EMPLOYED

A few words may not be amiss here concerning the methods employed by the writer in obtaining these microscopic characters. In general the method is that already described in a previous number of this journal.[1]

Obtaining spore prints.—In the case of fresh specimens just brought into the laboratory from the field, spore prints are very easily obtained by placing the specimens on a glass slide in such a manner that the tubes are in a perpendicular position so that the spores do not lodge on the sides of the tubes when they are liberated from the basidia. The slide with the fungus in position should be either wrapped in waxed paper or left over night or for several hours in the collecting

[1] Burt, E. A. *loc. cit.*

basket or other receptacle in which a fairly high humidity will be maintained, so that the liberation of the spores will not be prematurely stopped by the drying-out of the tissues of the fungus. If the specimens are dry when brought into the laboratory they may be moistened thoroughly with water and then treated as described above. One unaccustomed to this procedure will be surprised to find how large a percentage of the collections so treated will produce a good spore print. Specimens collected on the warm days that frequently come in January and February have often been treated in the above manner with gratifying and surprising results. When desiccation takes place by exposure to the air the vitality of many species is not destroyed. Buller[1] was able to restore normal vitality to such plants by placing wet cotton-wool on their upper surfaces. He was even able to revive the fruit bodies of *Daedalea unicolor* after they had been exposed to ordinary air at room temperatures for eight years and three months, and of *Schizophyllum commune* after an exposure of six years and three months. In most species, e. g., *Polyporus versicolor, P. hirsutus,* and *Lenzites betulina* the vitality was retained for a period of but two to three years.

Sectional preparations.—In case one is working with material that has been in the herbarium for several years the above method will not answer. Neither does it furnish any evidence as to the other microscopic characters of the plants. One must then resort to sectional preparations. These are cut free-hand with a very sharp sectioning razor. Free-hand sections are quickly made and the results from them are usually better than from microtome sections. It is impossible for the spores to retain their position on the basidia when subjected to the different processes involved in preparing material for microtome sectioning. The first requisite in successful free-hand sectioning is material in good condition; the second is a very sharp razor (preferably flooded with alcohol); the third is some little skill and experience. The hymenium of the specimen is first moistened with alcohol, then with water,

[1] Researches on fungi, pp. 105–111. 1909; and in Trans. Brit. Myc. Soc. 4: 106–112. 1913.

and a piece about 2 mm. square on the hymenial surface is cut out with a scalpel. If material is abundant the process may be reversed and a larger piece than needed may be cut out with the scalpel, trimmed to the requisite size, immersed in 95 per cent alcohol for a few seconds and then transferred to water. In the writer's experience the latter method is the more preferable and has probably been the one most used. The material does not soften while in alcohol, but that reagent is used only to facilitate the absorption of water by the tissue. Any rigidity that may be imparted to the tissue by the alcohol is probably overcome when the material is transferred to water. In some cases when this transfer is made the tissue either becomes very soft or very friable so that no razor, however keen, will cut a clean section through it. It is here that the latter method obtains preference over the former, for after some experience one can judge of the probable effect the water will have and by shortening the period that the material remains in the water the tissue is in better condition for sectioning.

The most instructive preparations are often those containing both longitudinal and cross-sections of the tubes. Such sections are easily obtained in one mount by cutting out the piece of material somewhat longer in one direction than stated above—say about 2×4 mm. on the surface. Several longitudinal sections may be cut from this and the position of the remaining bit of tissue so changed that cross-sections may be obtained.

For sectioning, the tissue is placed in the proper position in a piece of pith and as the sections are cut they may either be transferred directly to the slide by means of a camel's-hair brush dipped in alcohol, or they may be allowed to accumulate in the alcohol on the razor and then flooded off into a watch-glass containing alcohol. By the last method one can pick out with more accuracy the thinner sections by observing them under the lens of a low-power dissecting microscope. The writer has found it to be sufficient in most cases to transfer the sections directly to the slide, disregarding the thicker sections that are cut, or brushing them off the edge of the

razor with an outward stroke of the finger. The sections are placed in a drop of 7 per cent KOH solution on the slide. This immediately expands the hyphae of the tissue to their normal size. The KOH solution is then drained off and a drop of stain added.

Staining and mounting.—I have tested a considerable number of the more common stains and so far I have failed to find one that gives universally good results if the sections are to be made into permanent mounts. For temporary mounts there is nothing superior to a 1 per cent water solution of eosin, but when sections so stained are mounted in glycerin the color soon completely disappears. The same strength solution of alcohol eosin (in 95 per cent alcohol) often gave a good permanent stain but quite as often it, too, faded out in the course of several weeks, and when used it gives a precipitate that must be washed off with water before the cover glass is applied. Why this stain should remain permanent in some cases and not in others is a question that has not been answered. It may be due to the KOH that remains on the slide and in the sections, but flooding the sections with water after draining off the KOH solution did not seem to have any beneficial effect. Different strengths of alcohol were used in preparing the stain, but with alcohols weaker than 95 per cent the stain disappeared even more quickly and the precipitation obtained was so great that such stains were of no value. From the facts observed it seems more reasonable to suppose that the difference may be in the tissue of the fungus rather than in the stain or the glycerin. A solution containing equal parts of a 1 per cent water solution and a 1 per cent alcoholic solution of eosin gave no better results.

Magdala red, Congo red, neutral red, acid fuchsin, methylen blue, and saffranin T were used, and of these, only the last one gave a permanent stain and it has been used in a large part of the work. It is a rapid stain, though probably not quite so rapid as alcoholic eosin, and it is well to leave the stain on the sections for about one minute. A 1 per cent alcoholic solution was used, the stain being dissolved in 95 per cent alcohol. When a drop of this stain is added and drained

off, the sections must not be allowed to become dry or an orange precipitate is obtained that necessitates the addition of alcohol to dissolve it. This also dissolves the stain from the tissues and the sections must be restained. This precipitate is not formed if a little water is added to the stain after it is made up. This stain imparts a uniform dull red color to the tissue but the color brightens when glycerin is drawn under the cover glass. Since it is not a differential stain its use is not advised where only temporary mounts are desired. It gives best results with very thin sections or with sections in which the hyphae are loosely arranged.

After the cover glass is applied the sections are ready to be examined under the microscope, but if the saffranin T stain is used, it is better to place a drop of glycerin at one side of the cover glass, at the same time drawing off some of the surplus water from the opposite side by means of filter paper. Several slides of each species are retained and mounted in 66 per cent glycerin. After a week or more all traces of the glycerin are removed from near the outer edge of the cover glass by means of a soft cloth dipped in 95 per cent alcohol. The slides are then ringed with some suitable cement—gold-size being most often used—labeled, and filed away in order. It will usually facilitate subsequent examination of the slides if the spore characters for each species are written on a slip of gummed paper and glued to one end of the slide.

It is sometimes quite impossible to find spores in the sections treated in the manner outlined above, since they are often easily removed from the sterigmata and washed away before the cover glass is applied. To overcome this difficulty the writer sometimes finds it advisable to distribute between two slides the sections obtained, one slide to be treated as outlined above, the other to be mounted for temporary observation only. This last one should be stained with a water solution of 1 per cent eosin, a drop of the solution being added to the drop of KOH containing the sections. Sometimes the staining is unnecessary, especially if one is dealing with species which have colored hyphae and colored spores. A

preparation made in this manner will often show spores when other methods of demonstrating them have failed.

Even with the most careful manipulation one will sometimes fail to find the spores, and, indeed, some species seem to be almost always sterile. In the case of *Fomes fomentarius* I cut sections of all the specimens available, and only when as a last resort, I sectioned a small and very unpromising specimen did I find the spores. I have been able to locate them in but one of the few specimens of *Polyporus graveolens* that were available for examination.

As stated above, the literature dealing with American *Polyporaceae* contains many inaccurate observations concerning spores. This is due mostly to a lack of care in making sure that a given body in the hymenium is really the spore of the fungus in question. The writer is of the opinion that spores should not be recorded for a collection unless they are obtained from a spore print or are seen attached to basidia. The spores found on basidia are usually somewhat immature, at least as regards size, but from their shape one can judge whether the spores found free-floating in the mount have any relation to the species under consideration. Where such free spores alone are present there is always the possibility that they belong to some other fungus and they should not be taken into consideration unless present in large numbers. One must also guard against the fact that the cut ends of hyphae may be in such a position as to appear globose in form and such may be mistaken for spores.

Examining the context hyphae.—In obtaining the characters of the hyphae of the context a bit of tissue is picked out with the forceps and mounted on a slide in a drop of KOH solution. In the case of some of the species of the genus *Fomes* where the context is hard and woody, it is usually better to boil a bit of the context in a KOH solution for a few minutes. In this way the tissue is softened and when teased apart on the slide with needles, a cover glass added, and pressure applied, the hyphae will generally separate out so that their characters may be obtained. In all cases the

hyphal measurements given are for the hyphae in the context of the plant and not for those in the subhymenial tissue.

Statement of Problem

The writer presents in this paper the results obtained by carefully investigating some of the more common species of pore fungi, using the methods outlined on the preceding pages. There are certain groups of species in the *Polyporaceae* that are very much in need of just such treatment, and it is to these groups that the writer has turned his attention. The groups consist of closely related species that have been separated heretofore largely on external characters and in a great many cases the results have only led to confusion. The problem, as the writer saw it, was one involving a contribution toward a more exact characterization of these species and their separation, wherever possible or feasible, on some constant internal microscopic character. Some species are well enough marked by external characters so that such distinctions should be used only as supplementary characters, while in other cases the characters obtained by this study should displace those hitherto used.

The results obtained were not as gratifying as was expected when the work was undertaken. Only a small beginning has been made, for it is a laborious task involving the cutting and examination of many sections for each species in order to be sure that the characters shown by the first sections are constant for all collections of the same species. The work should be carried on although several years would be required for its completion. Permanent mounts of the sections have been made for each species and these are available for future reference. Criticisms and suggestions, both of methods employed and results obtained, are invited and will be given careful consideration.

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Polyporus abietinus Dicks. ex Fries and P. pargamenus Fries

P. abietinus was first described by Dickson,[1] in 1793, and appears to be almost cosmopolitan in its distribution. In the United States it is found wherever coniferous forests abound, from Canada to the Gulf of Mexico, and from the Atlantic to the Pacific Ocean. It is never found on the wood of deciduous trees, and as will be pointed out later, this fact affords almost the only constant character by means of which it can be separated from its near relative, *P. pargamenus.*

P. pargamenus was described by Fries,[2] in 1838, from plants collected on pine wood in Arctic America by the Franklin Expedition. The plant has not been reported from the western coast of the United States, but has been found in practically every state east of the Mississippi River, ranging west as far as Wisconsin, Kansas, Arkansas, and Colorado. It is also found in Europe. Most of the collections in this country under the name *P. laceratus* Berk., *P. xalapensis* Berk., or *P. ilicincola* Berk. and Curt., belong to this species. An examination of *P. pseudopargamenus,* as distributed by de-Thuemen,[3] shows it to be identical with *P. pargamenus.* The writer has not seen authentic specimens of the other species named above, but they are given as synonyms by Murrill.

By some writers the two species have been confused, due to the fact that the type specimens of *P. pargamenus* were reported as growing on the wood of coniferous trees, while in the United States the plant that has gone under the name *P. pargamenus* is confined entirely to the wood of deciduous trees. This has led some authors to regard the original *P.*

[1] Pl. Crypt. Brit. 3: p. 21. 1793.
[2] Epicr. Syst. Myc. p. 480. 1838.
[3] Myc. Univ. 1102.

pargamenus as probably a synonym for *P. abietinus*. In that event, the species on the wood of deciduous trees would have to be given another name. This point can be settled only by a study of the type specimens of *P. pargamenus*, if they are still preserved. Nearly all the exsiccati material has been distributed under the name *P. pargamenus*, and the plant is so common and the name so well established that it is the writer's opinion it should not be changed without recourse to the types.

The two species under discussion are very closely related and they are connected by intermediate forms to such an extent that it is difficult to refer some collections to their proper species. However, the usual form of the fructification is distinct enough. *P. abietinus* is usually much smaller, is frequently effused-reflexed with a narrow and often laterally continuous pileus, rarely more than 2 cm. in length, and the tubes sometimes break up into lamellae-like plates—a condition I have never found in *P. pargamenus*. That species often grows much larger than *P. abietinus*, sometimes attaining a length of 6–7 cm., and is often fan-shaped or cuneate in outline and attached by a narrow, attenuate, sometimes stem-like base, so that the form and size of the fruiting body will usually separate it from *P. abietinus*. The color, zonation, and pubescence of the pileus is similar in both species, though the pubescence is often inclined to be strigose in the latter plant and more velvety in the former. Both species often have a violaceous or lavender tint to the hymenium or on the margin of the pileus.

The microscopic appearance of the hymenium of the two species does not furnish additional characters for their separation. The spores are similar in size and shape, being cylindric or sometimes allantoid, hyaline, smooth, and measuring 5–7 × 1.5–2.5 μ (not globose, 4.5–5.5 μ as stated by Murrill). Murrill states that no cystidia are present in the hymenium of *P. abietinus* and to the writer's knowledge their presence has never been recorded. I have examined several collections of both *P. abietinus* and *P. pargamenus* and I find that the plants vary as regards this character. I am of the opinion that cystidia are probably always present, but at times are so rare

or so inconspicuous that close observation is necessary to detect them and I have often examined whole sections without being able to locate them. A similar section taken elsewhere in the hymenium may show an abundance of them. The accompanying illustrations (figs. 1 and 2) show the different forms they may assume, but perhaps the most common form is as shown in *a* of fig. 1. They are often scarcely larger in size than the basidia, but are different in shape, usually with the appearance of slender pegs tapering to a rather blunt point. Rarely they are somewhat fusiform in shape and reach a length of 20 μ and a thickness of 6 μ. These sizes are unusual, however. They are colorless or almost so, sometimes scarcely extending beyond the basidia, but sometimes projecting enough that one can easily pick them out with the low power of the microscope. They are usually unincrusted, but sometimes their tips are somewhat capitate with small crystals (see fig. 2). They are then much more conspicuous, and in some collections this appears to be the predominating condition.

Fig. 1. Section of the hymenium of *P. abietinus* showing cystidia and spores.

Fig. 2. Section of the hymenium of *P. abietinus* showing cystidia incrusted at the apex.

Before the writer had seen this more conspicuous type it was thought these sterile, inconspicuous structures might be basidia that had discharged their spores and had thus been rendered hyaline, as it is frequently found in other species that the mature spore-bearing basidia project somewhat beyond those that have not reached maturity. The shape of these bodies and the fact that they often assume a capitate apex, as do cystidia of many other species, make this view untenable. If more proof were needed it might be pointed out that these bodies are present in young specimens and in

the growing margins of mature specimens where it is evident that no mature basidia have yet been formed.

Neither can these structures be regarded as paraphyses that have become elongated and, therefore, more conspicuous. While there may be no ground for the belief that paraphyses can not assume such a form, yet there is no evidence to indicate that conspicuous sterile structures ever have arisen in such a manner. Moreover, the distribution of these structures under consideration makes impossible any such idea, as they are scattered promiscuously and do not alternate with the basidia.

These two species then are to be distinguished only by their habitat, and the size and shape of the pileus. In my own collecting experience the former character alone is enough to separate them, but when once the two plants are learned, the matter of form and size will usually be sufficient for the identification of the specimens, even if the habitat be unknown.

As stated above, the hymenium of *P. abietinus* may at times be lamellate. This statement is made only after a careful study of the facts in the case. They are as follows: There is a plant with apparently the same distribution as *P. abietinus*, in which the hymenium is entirely lamellate. No exactly intermediate conditions have ever been seen by the writer, though he has collected both forms in Colorado. In all other characters the two plants are precisely similar. The host is always the wood of coniferous trees; the pubescence and coloration of the pileus is the same; the spores and cystidia are similar; and the hymenium often has the violaceous tint characteristic of *P. abietinus*. *Irpex fusco-violaceus* is in all probability only another form of the same plant, although I have never seen specimens of that species with the well-marked lamellate hymenium of this form. The illustration (pl. 23 fig. 1) is from specimens communicated by Prof. C. R. Orton, of State College, Pennsylvania. He writes that the rot produced by this fungus is almost identical with the one produced by *P. abietinus*. Patouillard[1] represents the cystidia of *Irpex*

[1] Hym. Eur. *pl. 3. f. 23.* 1887.

fuscoviolaceus as incrusted at the apex in the same manner as shown in the accompanying illustration of *P. abietinus.* I have also found this condition to be predominant in the lamellate form of our species.

The following comparative synopsis of the two species discussed in this section is appended here:

1. Polyporus abietinus Dicks. ex. Fries.

Plate 23, figs. 1, 2.

Pileus coriaceous, *sessile or effused-reflexed,* 0.5–5 × 0.5–5 × 0.1–0.2 cm., white, cinereous, or blackish with age, villous, zonate; context not more than 1 mm. thick; tubes not more than 3 mm. long, the mouths white, bay, or violaceous, averaging 2–3 to a mm. in poroid forms, *but sometimes entirely lamellate;* spores cylindric or allantoid, hyaline, 5–7 × 1.5–2.5 μ; cystidia present or inconspicuous, hyaline, rarely incrusted at the apex, 3–6 μ in diameter, projecting 5–15 μ; hyphae of context hyaline, 3–4 μ in diameter.

On wood of *coniferous* trees, especially of *Pinus.*

Illustrations: Dicks. Pl. Crypt. Brit. 3: *pl. 9. f. 9.*—Fl. Dan. *pl. 1298, 2079. f. 2.*—Gill. Champ. Fr. *pl. 463.*—Swant. Brit. Fung. *pl. 33. f. 2–3.*

Specimens examined: Barth. Fung. Col. 3108.—Cooke, Brit. Fung. 512, 605.—Thuem. Myc. Univ. 6, 706.—Ell. N. Am. Fung. 8.—Ell. & Ev. Fung. Col. 303.—Krieg. Fung. Sax. 1205.—Rab.-Wint. Fung. Eur. 3235 (as *Irpex fuscoviolaceus*).—Rav. Fung. Am. 422; Fung. Car. I, 12.—Shear, N. Y. Fung. 307.—Mo. Bot. Gard. Herb. 4726, 4727, 4728 (Newfoundland), 3854, 4213 (New York), 4214 (Labrador), 4220 (Alabama), 4074 (Colorado).—Burt Herb. (collections from Vermont and Washington).—Overholts Herb. 2001 (Colorado), 2465 (Pennsylvania), 2472 (Maine).

2. Polyporus pargamenus Fries. Plate 23, fig. 9.

Pileus coriaceous, *sessile, often narrowed at the base,* 1–7 × 1–7 × 0.1–0.4 cm., whitish, cinereous, or brownish with age, villous or *velvety-pubescent,* zonate; context less than 1 mm. thick; tubes not more than 3 mm. long, the mouths white, bay, or violaceous, averaging 2–3 to a mm. in poroid

forms *but usually soon irpiciform;* spores cylindric or allantoid, hyaline, 5–6 × 1.5–2.5 μ; cystidia present or inconspicuous, hyaline, rarely incrusted at the apex, 4–5 μ in diameter, projecting 5–15 μ; hyphae of context hyaline, 4–5 μ in diameter.

On wood of *deciduous* trees.

Illustrations: Freeman, Pl. Dis. *f. 36.*—Hard, Mushrooms, *f. 345.*

Specimens examined[1]: Barth. Fung. Col. 2825, 2924 (as *Coriolus prolificans*).—Ell. N. Am. Fung. 312.—Ell. & Ev. Fung. Col. 302.—Rav. Fung. Am. 423, 108 (as *Irpex fusco-violaceus*).—Rav. Fung. Car. I, 13.—Rab.-Wint. Fung. Eur. 3331.—Shear, N. Y. Fung. 38.—Thuem. Myc. Univ. 1102 (as *P. pseudopargamenus*).—Mo. Bot. Gard. Herb. 4086 (Missouri), 4431 (Arkansas), 3855 (New York), 4443 (Indiana), 4439 (Kentucky), 4433 (Illinois), 4436 (Alabama), 4559 (Georgia), 4557 (Florida), 42875 (New Hampshire).—Burt Herb. (collections from Pennsylvania, Vermont, Kansas, and Massachusetts).—Overholts Herb. 476, 269, and others (Ohio), 1756 (Colorado).

Polyporus adustus Willd. ex Fries, P. fumosus Pers. ex Fries, P. fragrans Peck, and Related Species

Perhaps no species have been more confused in American mycology than these three, together with a few other closely related forms both of Europe and America. They all agree in the one character of having a hymenium that usually becomes more or less smoke-colored at maturity. In *P. adustus* and its closest relatives, *P. crispus* Fries and *P. Burtii* Peck, the hymenium is usually black or grayish black from the first, while in *P. fumosus* and *P. fragrans* it frequently becomes

[1] Ell. & Ev. Fung. Col. 804, distributed as *P. pargamenus*, is *P. hirsutus* (certainly not *P. pubescens* as stated by Lloyd, Letter No. 52, p. 20). Ell. & Ev. N. Am. Fung. 1934, distributed as *P. pargamenus*, is not this species. The appearance of the plant suggests a form of *Irpex tulipifera*. I have made a microscopic study of the hymenium of the specimen and I find it has the larger incrusted cystidia of that species and not the inconspicuous cystidia of *P. pargamenus*. Mycological literature contains several names for plants closely related to, if not identical with, *Irpex tulipifera* and until the limits of the species are better known the writer hesitates to refer the above specimen with certainty.

darker in mature plants but often remains white, sometimes assuming an ochraceous tint in herbarium specimens.

Of the above-named species, the first three have been referred to *P. adustus* by Murrill. *P. adustus* was described by Willdenow[1] in 1787. *P. crispus* was first described as a species by Persoon,[2] in 1799, and was later (1815) accepted by Fries[3] and so maintained by him in his 'Hymenomycetes Europaei.' *P. Burtii* was described from Vermont by Peck,[4] in 1897, and has not since been reported. *P. fumosus* was first described by Persoon,[5] in 1801, and *P. fragrans* by Peck,[6] in 1878. There are several other names for plants closely related to, if not identical with, these species but the writer has had no opportunity to study them. One of these, *P. subcinereus,* described by Berkeley, in 1839, is said to have been repudiated by its author and the plants referred to *P. adustus. P. Halesiae* Berk. & Curt.[7] is probably distinct, and *P. Lindheimeri* Berk. & Curt.[8] is not at all related to *P. adustus,* as stated by Murrill, but is a large-pored species with a brown context.

In working over the collections referred to *P. adustus* in the herbarium of the Missouri Botanical Garden, the herbarium of Dr. E. A. Burt, and the writer's herbarium, it became evident that we are here concerned with a species that has been used as a sort of dumping-ground for all plants with a black hymenium and a rather thin context, while plants of thicker context and lighter-colored hymenium have been referred to *P. fragrans* or to *P. fumosus,* according to whether a pleasant odor was or was not noticed in the plants. Such procedure has resulted in the bringing together of a heterogeneous mass of material under the name *P. adustus.* This material was very readily separated into three fairly distinct sections besides the collections that properly belonged under

[1] Fl. Berol. p. 392. 1787.
[2] Persoon, C. H. Obs. Myc. 2: p. 8. 1799.
[3] Fries, E. Obs. Myc. 1: p. 127. 1815.
[4] Bul. Tor. Bot. Club 24: p. 146. 1897.
[5] Syn. Fung. p. 530. 1801.
[6] Rept. N. Y. State Mus. 30: p. 45. 1878.
[7] Grev. 1: p. 52. 1872.
[8] *Ibid.* p. 50. 1872.

P. fumosus. After considerable study the writer has decided that to *P. adustus* should be referred those collections with a thin, finely tomentose pileus, a thin, even margin, and minute black pores. The species does not grow densely imbricate as in *P. crispus* (see pl. 23 fig. 7) and does not have the crisped margin of that species. The illustration of *P. adustus* given by Patouillard[1] represents our plant very well. From *P. Burtii* it is to be distinguished by the smaller and more equal pores, the thinner, sterile margin of the pileus, and the firmer context. It is much more abundant than the other three species and frequently grows semi-resupinate.

According to Fries, *P. crispus* differs from *P. adustus* in having a thin, crisped, margin and large unequal pores. One lot of segregates from my *P. adustus* material possesses just those distinguishing characters, and I have, therefore, revived the Friesian name and applied it to my plants. They are certainly distinct from the specimens referred to *P. adustus* though connected by intergrading forms to some extent. The illustrations (pl. 23 figs. 7 and 8) show typical specimens of the two species.

I have seen no specimens other than the types that could be referred to *P. Burtii.* The type specimens differ from the above conception of *P. adustus* in having a somewhat thicker context, a thicker margin that is fertile below, and larger and more unequal pores. The hymenium is black, as in that species, and the surface of the pileus is finely tomentose. The flesh of the pileus is also very soft and almost floccose in texture. It has been held by some that the mouths of the tubes in *P. adustus* become larger and more irregular in mature plants, and if such a character stood alone in the differentiation of these forms it probably should not be considered a specific character. But it is the writer's opinion that in *P. adustus* they do not become much larger in old plants, and since *P. Burtii* differs also from that species in the other characters mentioned above, we must consider it a valid species, at least until other collections throw more light on the subject. From *P. crispus* it may be separated by the fact that the

[1] Tab. Anal. Fung. *f. 142.*

margin is not crisped, sterile, and thin, that the pubescence of the pileus is not nearly so prominent, and that the context is soft and floccose. The type specimens are not densely imbricate as in *P. crispus* but more nearly approach the condition found in *P. adustus.*

The microscopic characters of these three species are identical and do not afford additional means of separating them. The tramal tissue of the pores is decidedly brown in color, the hyphae are small, and a large percentage of them are cut transversely in a cross-section of the hymenium. The spores in all three species are oblong or oblong-ellipsoid, and measure 3.5–4.5 × 1.5–2.5 μ. There are no cystidia or other sterile bodies in the hymenium.

In endeavoring to find characters on which to separate the three above-named species (and especially *P. adustus*) from specimens heretofore referred to *P. fumosus* and *P. fragrans,* recourse was had to microscopic sections of the hymenium. It was at once apparent that when longitudinal sections were prepared, according to directions given on page 678 of this paper, the tramal tissue of the tubes of *P. adustus, P. crispus,* and *P. Burtii* were decidedly brown in color, while those of *P. fumosus* and *P. fragrans* were entirely hyaline, except for the eosin stain. This character has been tested out thoroughly and is believed to be a satisfactory and constant one on which to differentiate these two groups of species. By obscuring the labels on the slides containing the sections of the different species it was found possible to easily separate the sections of the species of the one group from those of the other group by this character, and then verify the separation by uncovering the labels. Since suitable sections can be readily prepared in a very few minutes, the task of deciding between the two groups is an easy one when they cannot be readily separated on the general appearance of the specimens. Some such method of procedure is especially desirable in separating *P. adustus* from *P. fumosus,* since thin or young specimens of the latter are easily confused with the former species. However, care must be taken not to confuse the dark color sometimes obtained in thick sections of *P. fumosus* with the truly

brown color of the hyphae in *P. adustus*. In the hyphae of the latter species the color is brown, whether the sections are thick or thin. This test will usually apply to cross-sections of the tubes as well as to longitudinal sections, except that when the hymenium of a growing specimen is bruised, dried, and then sectioned, the mouths of the tubes and the hyphae at the ends of the tubes often show a brownish discoloration that may be confusing. *P. crispus* and *P. Burtii* usually are easily distinguished without this test, but the results are even more marked in the case of those two species than in *P. adustus*.

When Peck first described *P. fragrans* he stated that it was closely related to *P. fumosus,* but differed in having unequal pores and an agreeable odor. In a later report he remarked that it should perhaps be considered a variety of that species. Microscopically the two plants are the same. There are no cystidia and the spores are oblong-ellipsoid, and measure 4.5–6 × 2–3 μ, thus being slightly larger than the spores of the three species discussed above. The spore characters given for both species in the 'North American Flora' are erroneous. From our present knowledge of the variability of odors in the fungi[1] we are not warranted in laying much stress on the fragrant odor ascribed to *P. fragrans*. Bresadola[2] discusses *P. fumosus* under the name *P. imberbis* and states that the plant at times has a subanise odor. I have never obtained such an odor from plants heretofore referred to that species, but frequently the plants do have an odor that I would not describe as pleasant. In the face of such evidence, it seems reasonable to conclude that the odor alone should not separate the two species in question. As to the size and regularity of the pores of the two species, I find collections of *P. fumosus* in which the younger specimens have minute pores and the older ones have large and irregular pores, and collections of *P. fragrans* with both large and small pores. I conclude,

[1] e. g., *Polyporus gravcolens* Schw. I have collected this species several times and have had growing plants under observation for three seasons and at no time have I been able to obtain the slightest trace of an odor that would warrant the application of "sweet knot" to that species. Similar results have been reported by others. There is good authority, however, for stating that it is at times very fragrant.

[2] Fung. Trid. p. 29.

therefore, that we are here dealing with a character that varies with the age of the plants or even varies in different plants of approximately the same age. In other characters the two species are identical. Bearing in mind then the following points: (1) Peck's admission concerning his species, (2) the little reliance that is to be placed on odors in at least some of the fungi, (3) the evidence that *P. fumosus* is sometimes fragrant as it grows in Europe, and (4) the variability in the size of the pores in a single collection, we can only conclude that *P. fragrans* is at most only a form of *P. fumosus* and not worthy of a distinct name.

There are a few other names that need to be mentioned before dismissing this group of species. *P. salignus* Pers. ex Fries is generally held to be *P. fumosus,* and Fries' illustration[1] certainly agrees with the species as it grows in this country. *P. Holmiensis* Fries, as distributed by Romell,[2] is surely our plant and it is so regarded by Bresadola. *P. imberbis* Bull. ex Fries, as represented by Bresadola, is the same plant, but the name was not recognized by Fries in his 'Systema Mycologici' and so cannot be used for our plant.

The following key will aid in distinguishing the four species presented here:

Pileus rather thin; hymenium black or smoky black; tramal hyphae distinctly brown in section 1

Pileus thicker; hymenium pallid to somewhat smoky; tramal hyphae hyaline or nearly so in section ..... *4. P. fumosus*

1. Pileus finely tomentose; margin thin, even, sterile below; context firm when dry; pores minute; plants slightly, if at all, imbricate..*1. P. adustus*

   Pileus adpressedly fibrillose on the margin, usually strigose toward the base; margin thin, crisped or wavy, sterile below; context firm when dry; pores larger and unequal; plants usually closely imbricate ..... *2. P. crispus*

   Pileus finely tomentose; margin acute but thicker than in the preceding species, even, fertile below; context soft and floccose; pores unequal; plants scarcely imbricate..... *3. P. Burtii*

1. Polyporus adustus Willd. ex Fries. Plate 23, fig. 8.

Pilei *not much imbricate* though somewhat so at times, 1–6 × 3–8 × 0.1–0.6 cm., white to smoky white or pale tan, rarely with reddish blotches or zones, *finely tomentose to short villous-tomentose,* zonate or azonate; margin *thin, even,*

[1] Ic. Hym. 2: *pl. 181.*

[2] Fung. Scand. 11.

often black in dried specimens, sterile below; context white or pallid, firm and corky when dry, 1–4 mm. thick, in large specimens separated from the hymenium by a narrow dark line; tubes less than 2 mm. long, the mouths *grayish black to black, scarcely visible to the naked eye, averaging about 6 to a mm.;* tramal tissue decidedly *brown in color under the microscope;* spores oblong or oblong-ellipsoid, rarely slightly curved, smooth, hyaline, 3.5–5 × 1.5–2.5 μ; cystidia none.

On dead wood of deciduous trees.

Illustrations: Pat. Tab. Anal. Fung. *f. 142.*—Rostk. in Sturm's Deutsch. Fl. **3**: fasc. 16. *pl. 38.*

Specimens examined: Cooke, Fung. Brit. 2.—Ell. N. Am. Fung. 6.—Ell. & Ev. Fung. Col. 206.—Krieg. Fung. Sax. 1319. —Rabenh. Herb. Myc. 412.—Rav. Fung. Am. 421.—Shear, N. Y. Fung. 32.—Mo. Bot. Gard. Herb. 4222 (Newfoundland), 4223 (New York), 3851 (Missouri).—Burt Herb. (collections from Vermont, Ohio, Massachusetts, and New York).—Overholts Herb. 284 (Ohio), 572 (Missouri), 2239 (New York), 1780 (Colorado), and others.

2. **Polyporus crispus** Pers. ex Fries. Plate 23, fig. 7.

Pilei *more or less densely imbricate and overlapping,* 2–7 × 1–5 × 0.1–0.4 cm., gray to avellaneous, sometimes cinnamon to clay-colored in herbarium specimens, *adpressedly fibrillose toward the margin, usually strigose toward the base,* zonate or azonate; *margin very thin, radiate-lineate, crisped or wavy,* often becoming black, sterile below; context white or pallid, often brownish in herbarium specimens, soft and fibrous to corky, 1–3 mm. thick, usually separated from the hymenium by a narrow dark line; tubes 1–3 mm. long, the mouths *grayish black to black, unequal, irregular, averaging 3–6 to a mm.;* tramal tissue decidedly brown in color under the microscope; spores oblong or oblong-ellipsoid, smooth, hyaline, 3.5–4.5 × 1.5–2.5 μ; cystidia none.

On dead wood of deciduous trees.

Illustrations: Fl. Dan. *pl. 1850.*

Specimens examined: Romell, Fung. Sax. 8 (as *P. adustus*).—Thuem. Myc. Univ. 604 (as *P. fumosus*).—Mo. Bot.

Gard. Herb. 42868, 42848 (Arkansas), 4180 (Missouri).—Overholts Herb. 386 (Indiana), 105 (Ohio).

3. Polyporus Burtii Peck. Plate 23, fig. 4.

Pilei not closely imbricate, 1–2.5 × 2–5 × 0.3–0.5 cm., gray or pinkish buff, *finely tomentose,* azonate; margin *acute but rather thick, deflexed, even, concolorous, fertile below;* context *soft and sub-floccose* in dried plants, 2–4 mm. thick; tubes 1–2 mm. long, *the mouths grayish black to smoky black,* unequal, irregular, *averaging 2–4 to a mm.;* tramal tissue decidedly brown in color under the microscope; spores oblong-ellipsoid, smooth, hyaline, 4–4.5 × 1.5–2 μ; cystidia none.

On stump of yellow birch. Known only from the type locality, Middlebury, Vermont.

Specimens examined: Burt Herb. (type collection).

4. Polyporus fumosus Pers. ex Fries. Plate 23, fig. 3.

Pilei simple or imbricate, 2–10 × 3–15 × *0.5–2 cm.,* white to ochraceous or smoky white, sometimes stained with reddish, *finely tomentose to glabrous, sometimes with a rather broad, marginal furrow;* context white or pallid, soft corky to woody when dry, *2.5–10 mm. thick,* usually zonate, always separated from the hymenium by a narrow dark line, *anise-scented or with a disagreeable odor;* tubes 1.5–4 mm. long, the mouths *white to grayish black, usually becoming black when bruised,* averaging 3–4 to a mm.; tramal tissue hyaline or nearly so under the microscope; spores oblong-ellipsoid, smooth, hyaline, 4.5–6 × 2–3 μ; cystidia none.

On dead wood of deciduous trees, especially elm.

Illustrations: Fries, Ic. Hym. *pl. 181* (as *P. salignus*).—Bres. Fung. Trid. *pl. 135* (as *P. imberbis*).—Massee, Brit. Fung. Fl. *f. 14–15.*—Rostk. in Sturm's Deutsch. Fl. **3**: fasc. 16. *pl. 42.*

Specimens examined: Ell. & Ev. N. Am. Fung. 2902.—Shear, N. Y. Fung. 31.—Thuem. Myc. Univ. 5.—Mo. Bot. Gard. Herb. 43648 (Missouri), 4277 (Kansas).—Overholts Herb. 455, 527 (Ohio), 436 (Canada), 370 (Indiana), and others.

The White Species of Polyporus — Those Watery and Fleshy-Tough when Fresh and with White Context and Spores

This group of plants has probably been the source of more trouble and exasperation to those collecting them than any other group in the *Polyporaceae*. Collectors have sent them to various mycologists for determination, and quite often no two will agree on the name that should be applied to any one form.

The group of species with which we are here concerned has been divided into two genera by Murrill, namely, the genus *Tyromyces* and the genus *Spongipellis*. Since the characters that separate the latter from the former genus are not always well defined, it would seem better had they been united into one genus. The group includes those species found only during the summer and fall, growing on logs or on living trees, and further characterized by being white or whitish throughout, and having a more or less watery and soft fibrous context. Some of the species have characteristic odors that will usually aid in their identification. When dry the context of some of these is soft and friable, sometimes more solid, and sometimes differentiated into an upper soft portion and a lower firm portion. We cannot include here all of the species referred by Murrill to the two above-named genera, partly because there has been no opportunity to study all of them and partly because many of them are limited in their distribution and are only infrequently found by collectors. Those that are of common occurrence in the Ohio and the upper Mississippi River valleys have been studied and the results here presented. The series thus limited includes the following species: *P. albellus* Peck, *P. caesius* Schrad. ex Fries, *P. chioneus* Fries, *P. delectans* Peck, *P. fumidiceps* Atk., *P. galactinus* Berk., *P. lacteus* Fries, and *P. spumeus* Sow. ex Hornemann. These are not all closely related and most of them are not difficult to determine but they have been more or less confused in this country, and their distinguishing characters are here pointed out.

P. chioneus, P. albellus, and P. lacteus.—*P. chioneus* was described by Fries,[1] in 1815. In his 'Hymenomycetes Europaei,' published in 1874 (p. 546), he described it somewhat more fully as follows: "Albus, pileo carnoso, molli, laevigato, azono, postice saepe porrecto, margine inflexo; poris curtis, exiguis, rotundis, aequalibus, integerrimis. . . . . Ad truncos v. c. *Betulae*. . . . . unciam latus, odore acido." In 1878 Peck[2] described *P. albellus* from New York, also growing on birch. Peck evidently was not acquainted with *P. chioneus,* but he regarded his species as probably more closely related to *P. paradoxus* Fries and *P. betulinus* Bull. ex Fries. The only points of difference in the descriptions of *P. albellus* and *P. chioneus* are: (a) in size, Peck's species being described as "two to four inches broad, one to one and a half thick," and (b) in pubescence, the pileus being "smooth or sometimes slightly roughened by a slight strigose tomentum." Both descriptions mention the soft context, white color, and "acid" odor. Saccardo[3] has listed *P. albellus* as a synonym for *P. betulinus,* and while the general form and size of the two species is at times somewhat similar, it does not require close observation to distinguish them. The same cannot be said of *P. albellus* and *P. chioneus.* Murrill[4] has listed them as synonyms and the writer has expressed the same opinion in a recent paper.[5]

P. lacteus may well be brought into the discussion at this point. It was described in 1821. The description and figure[6] call for a plant similar in size and habit to *P. chioneus* but differing from that species and from *P. albellus* in having a decidedly pubescent pileus and a lacerated and labyrinthiform hymenium. These characters should be sufficient to separate at once *P. lacteus* from the other two species, and the writer can neither accept nor understand the determinations of those who would refer our common plant with a glabrous pileus and

[1] Obs. Myc. **1**: p. 125. 1815.
[2] Rept. N. Y. State Mus. **30**: p. 45. 1878.
[3] Syll. Fung. **6**: p. 139. 1888.
[4] N. Am. Fl. **9**: p. 35. 1908.
[5] Ann. Mo. Bot. Gard. **1**: p. 97. 1914.
[6] Fries, E. Ic. Hym. **2**: *pl. 182. f. 1.*

even hymenium to *P. lacteus*. Romell,[1] after a short description of *P. lacteus* as he understands it, says:

> "This species seems to be identical with one known in America as *Polyporus chioneus*. . . . . My specimens agree with the authentic specimens of *P. lacteus* at Kew. In Fries' herbarium neither *P. lacteus* nor *P. chioneus* is represented by authentic specimens as far as I know. There is, however, a collection referred to *P. chioneus* by Robert Fries, and this collection differs from my plant not only by the *glabrous surface of the pileus* but also by having the hyphae substantially *parallel and simple*. . . . . ." (Italics are the writer's.)

Fig. 3. Hyphae of *P. chioneus*.

It is unfortunate if, with the easy access to Fries' description, American mycologists of repute have sent specimens of a pubescent *Polyporus* to Europe under the name, *P. chioneus*. On the other hand, if the determination were that of an amateur it should not have been seriously considered by Mr. Romell. Whichever may have been the case, it is the writer's opinion that such determinations are the exceptional ones and not the rule, for the plant that is usually referred to *P. chioneus* (including *P. albellus*) is usually, if not always, entirely glabrous and has even tube mouths. In fact, it is the writer's opinion that *P. lacteus* and *P. chioneus* have been less confused in this country than in Europe. If there has been a tendency to confuse *P. lacteus* with anything it is with *P. galactinus*, as I have found several collections so misdetermined. The important point of the extract from Romell's paper is, however, that the collection to which reference is there made as having a glabrous pileus and simple hyphae in the context, in all probability represents the species that is interpreted in this paper as *P. albellus*.

Having fixed upon the distinguishing characters of *P.*

[1] Hym. Lapp. p. 15.

chioneus and accepting Fries' idea of *P. lacteus,* it becomes an easy matter to differentiate between *P. chioneus* and *P. albellus.* As stated above, and as will be seen in the accompanying illustration (fig. 4), the hyphae in the context of *P. albellus* are unbranched or at most very infrequently branched, while those of *P. chioneus* (fig. 3) are branched to a very great degree, and they vary considerably in size, some being narrow (5–6 μ) and others twice as thick. This is not the only distinguishing character, nor the one that was first hit upon by the writer, although it is probably the most reliable. The relative thinness of the pileus in proportion to its length is a distinguishing character of *P. chioneus.* In other words, the pileus is usually thin and spreading in *P. chioneus,* while in *P. albellus* it is thicker, convex or ungulate, and triangular in section. This is only a general statement of a character that varies considerably. An additional character is found in an examination of a cross-section of the hymenium, though the sections must be cut very thin to see it at its best. In sections of *P. albellus* the hyphae in the trama of the pores appear to run in all directions and give a peculiar, ever-changing appearance as they are viewed at changing foci. They are also all of one size. In *P. chioneus* the hyphae in the trama of the pores all run in one direction and practically all are cut transversely in a cross-section of the hymenium. The trama is seen to be made up of a background of a pseudocellular structure, with minute openings that indicate the cavities of the closely compacted hyphae. Interspersed over this background one sees cross-sections of hyphae two to three times larger, and standing out much more plainly than the sections of the compact hyphae in the background. It was at first thought these

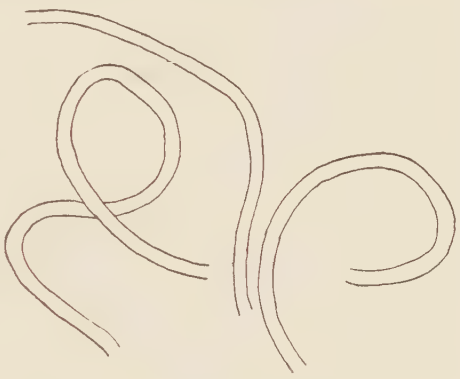


Fig. 4. Hyphae of *P. albellus.*

larger hyphae might belong to some other fungus living within the tissues of this species. This supposition is rendered improbable, however, by the fact that they are invariably present in all collections, and that while other fungi frequently attack all of these white species, their hyphae are invariably much smaller than those of the fungi attacked.

The evidence seems very clear, however, that these two species should be considered as distinct. When once differentiated they can usually be separated on the basis of their general habit, without recourse to the character of the branched or unbranched hyphae in the context, though that character can always be relied upon in establishing beyond a doubt the identity of the species. In other characters the two species are very similar. Both are glabrous or practically so; are covered with a thin grayish or yellowish pellicle that becomes more evident when the plants are dried; have a sweet acid odor when fresh, a soft and friable context when dry; and the spores are the same, being cylindric, often slightly curved, and measuring 3–4×0.7–1.5 μ. There are no cystidia.

There is considerable doubt in the writer's mind as to whether the true *P. lacteus* occurs in this country. There is a collection in the herbarium of the Missouri Botanical Garden and another in the writer's herbarium that should perhaps be referred to that species, but the hymenium has been disorganized by the growth upon it of another fungus, so that no spores are present. If future collections should show that the spores are similar to those of *P. chioneus*, the plants should in all probability be referred to *P. lacteus*. The pileus is somewhat strigose or fibrillose-pubescent, though the mouths of the tubes are not labyrinthiform. The pileus is too pubescent for either *P. chioneus* or *P. albellus* to which latter species the plants were once referred by Lloyd. It is possible that they represent *P. lacteus* as more recently defined by Lloyd.[1] I have seen no specimens so referred by him and his description of the plant as "a common white species" and again as "a frequent plant" throws some doubt on my opinion, for the plant is a rare one.

[1] Letter No. 49, p. 14.

According to the writer's notes on specimens of *P. lacteus* in the herbarium of the New York Botanical Garden, that species, as it appeared in the 'North American Flora,' is *P. albellus* as here defined, at least in part. Neither can the writer accept Romell's interpretation of *P. lacteus,* but if such a plant exists it must agree in the main with Fries' description and figure, and neither of the above interpretations do so agree. I do not know what Bresadola's latest ideas on the subject are, but at one time he regarded *P. lacteus* and *P. chioneus* as synonyms—a position just as untenable as that taken by Murrill and Romell.

According to the above interpretation of *P. chioneus* and *P. albellus,* the presentation of the two species in a recent paper[1] by the writer should be modified, and those collections that show simple hyphae in the context should be referred to *P. albellus* and those with branched hyphae should be referred to *P. chioneus.*

P. delectans and P. spumeus.—The first one of these species was described by Peck,[2] in 1884, from specimens collected in Ohio by Morgan. It is a large or medium-sized plant and was described as having a fleshy-fibrous context, a glabrous or floccose-tomentose pileus, and long tubes with large unequal mouths. By this last character and by the large size of the plant and the ellipsoid or subglobose spores it is easily distinguished from the species discussed above. In size of pores and length of tubes it is intermediate between the above species and *P. obtusus* Berk. A much more closely related species, however, is *P. spumeus.* The original notes of Sowerby on this species are very meager. The plant is described as "oozes from decaying elms in a very soft frothy mass, hardening in a day or two; and if it dries favorably, the pileus becomes hispid. The pores are small and nearly round; the tubes not long." In Sowerby's text[3] this species is followed by *P. betulinus.* Plates 211 and 212 are cited as representing the two species, respectively. Plate 211 shows a

[1] *loc. cit.* p. 97.
[2] Bul. Tor. Bot. Club 11: p. 26. 1884.
[3] Colored Figs. Eng. Fung. *pl. 211–212.* 1797–1803.

plant with a substipitate base, an incurved margin, and short tubes. One figure shows the plant from a front-underneath view, the other shows half of the plant with the cut surface outward and the hymenium upward. Plate 212 shows practically the same thing but with a little more detail, and it is a fair representation of *P. betulinus*. All later descriptions of *P. spumeus* are either based entirely on pl. 211, or else on plants that have no resemblance to the one that has since been referred to *P. spumeus*. Fries' description[1] says: "basi stipitiformi, margine incurvato."

This gives us but two alternatives from which to choose. Either Sowerby confused his illustrations of *P. spumeus* and *P. betulinus* and inserted two plates of the same species (*P. betulinus*), or else there existed at that time a plant closely related to *P. betulinus* but growing on elm and thought by Sowerby to be distinct. Since the mutual resemblance of Sowerby's two plates is so great, it is the writer's opinion that he had drawn two plates of *P. betulinus* and by mistake inserted both of them instead of one of that species and one of *P. spumeus*. This theory is borne out by the fact that he makes no mention of a stipe-like base nor an incurved margin to the plant. We may also conclude that Fries' description was drawn, in part at least, from pl. 211, for it is inconceivable that with access to Sowerby's figure he would have referred to that species a plant that departs so widely from the authentic illustration, unless he was also of the opinion that pl. 211 was a mistake.

This mistake (for so it seems we must regard it) has caused some little confusion in the literature. Fries' idea of *P. spumeus* was evidently gained, in part at least, from Sowerby's plate, for he refers as a synonym for *P. spumeus*, *Boletus suberosus* of Wahlenberg[2]. But Wahlenberg was aware of the existence of a *Boletus suberosus* of Linnaeus[3] and expressed the doubt that his species was the same as that one. *Boletus suberosus* of Linnaeus has always been regarded as

[1] Hym. Eur. p. 552. 1874.
[2] Fl. Upsal. p. 457. 1820.
[3] Sp. Plant. p. 1176. 1753.

a synonym for *P. betulinus*. In 1823 Hornemann[1] published a figure of *P. spumeus* entirely different from Sowerby's original figure, but in all probability a better representation of his original species. It was not, however, so accepted at the time. In the text accompanying the plates in 'Flora Danica,' Hornemann refers to Sowerby's original figure as a variety (var. *stipitatus*) of *P. spumeus*. This was evidently only a makeshift to dispose of a troublesome figure, and since the figure itself was evidently an error, Hornemann's disposition of it need have no weight. Subsequent writers did not concur in his opinion, however, and the confusion was only made worse, for now some regarded that there were two distinct plants passing under the name of *P. spumeus*. In Hooker's 'English Flora,'[2] in which the fungi were written up by Berkeley, both Hornemann's and Sowerby's illustrations are cited as representing *P. spumeus,* and Hornemann's figure is given priority in the order of citation. Again the plant is described as possessing an obsolete stipe and an incurved margin—characters either taken from Sowerby's illustration or copied from Fries. That Berkeley was in doubt as to the correctness of Sowerby's plate is evidenced by the statement: "According to Fries, the figure of Sowerby represents the species in an imperfect state. . . . . ." In 1874 Fries[3] accepted Sowerby's figure as representing *P. spumeus* and referred Hornemann's figure to *P. epileucus*. This reference was evidently followed by Saccardo. Berkeley[4] published an illustration of *P. spumeus* that corresponds well with Hornemann's figure and agrees with the plants since referred to that species. Thus there has arisen an interesting situation in which, according to the writer's interpretation, a well-known species is referred to an erroneous illustration that cannot possibly represent it, while the authentic illustration is referred to another species. Of course it is possible that Hornemann may have misinterpreted Sowerby's *P.*

[1] Fl. Dan. *pl. 1794*. 1823.
[2] Eng. Fl. 5²: p. 139. 1836.
[3] Hym. Eur. p. 552. 1874.
[4] Outl. Brit. Fung. *pl. 16. f. 4*. 1860.

spumeus, in which case the name should be written *P. spumeus* Hornemann, Fl. Dan. *pl. 1794.* 1823, since there is no doubt that Hornemann's figure represents *P. spumeus* as it is known in Europe to-day. But the writer prefers to accept Hornemann's plate as a correct interpretation of Sowerby's species (disregarding pl. 211) and write the name as *P. spumeus* Sow. ex Hornemann. If the writer's theory is correct, there never existed a plant, the name of which could be written as *P. spumeus* Sow. ex. Fries, Syst. Myc. 1: 358. 1821,[1] since Fries never illustrated the plant, and his descriptions, several times repeated, were based, in part at least, on the erroneous pl. 211 of Sowerby.

In the American literature the plant was first described by the writer in a recent paper.[2] The relation of Sowerby's figure to the species was not then understood and the statement was there made that "the plants so referred do not agree with the figure given by Sowerby, nor with Fries' description." There are but few references to its occurrence in this country, although it is a fairly common species. Lloyd reports receiving it from several widely separated localities.

Whether others may agree with the writer or not, the evidence here presented should at least have the effect of doing away with the inconsistency of citing both Sowerby's illustration and that of Hornemann as representing the same species.

P. spumeus is not likely to be confused with any species except *P. delectans.* These two intergrade to some extent. The former species has a strigose-tomentose surface to the pileus while the latter is glabrous or only slightly tomentose. Heavy rains or a little handling of the plant may cause the pubescence on *P. spumeus* to become matted and appressed, but when specimens are found growing imbricated so that the lower pilei are protected by the ones above, the character is very marked. The tubes in both species are long and slender, but in *P. delectans* the mouths are larger and more sinuous, usually measuring 0.5–1 mm. in diameter, while those of *P. spumeus* are smaller, measuring about 3–4 to a mm., and col-

[1] cf. Ann. Mo. Bot. Gard. 1: p. 99. 1914.
[2] *loc. cit.*

lapse when dry. This collapsing is due to the thinness of the dissepiments—a character easily made out in transverse sections of the hymenium. The illustration (pl. 24 fig. 14) shows the larger tubes of *P. delectans*. The spores of the two species are practically the same, varying from ellipsoid to ovoid or subglobose, and measuring 5–6×4–5 μ. They are frequently guttulate in both species. There are no cystidia in the hymenium.

P. galactinus.—This species is a fairly well-marked one and only its distinguishing features will be pointed out here. It was originally described by Berkeley from specimens collected in Ohio by Lea. It is eastern in its range in the United States, occurring from Maine to Missouri and probably no farther south than West Virginia. There are but three common plants in this section of *Polyporus* that possess characteristic odors when fresh and growing. *P. galactinus* is one of them. The odor is usually described as "acid," but to the writer it is a very pleasant and fragrant odor, but not persisting in the dried plants. Characters are not wanting to separate this species from the group just discussed in this section. The pileus is strigose-pubescent, as shown in the illustration (pl. 24 fig. 15), the tubes are very small, and the spores are minute, ellipsoid or subglobose, uninucleate, and measure 3–4 × 2–3 μ. From *P. delectans* and *P. spumeus* it may be separated by the minute pores and the smaller spores. From *P. fumidiceps* Atk. it differs in the decidedly pubescent pileus and larger size. From *P. caesius*, which it resembles in its hairy covering, it differs in its larger size and ellipsoid spores. There are no cystidia.

P. caesius.—This species has long been recognized as a well-marked one, characterized by the villous-strigose pubescence on the pileus, the bluish or grayish blue tint often present on the hymenium, and the minute, cylindric, curved spores. From *P. galactinus* it is separated by its small size and different spores; from *P. chioneus* and *P. albellus* by the pubescent pileus; from *P. lacteus* by the more strigose pileus and the unbranched hyphae of the context.

P. fumidiceps.—This species was described by Atkinson[1] in 1908, and has not since been reported. Since the writer finds it to be a rather common species in Missouri, and since a description has not appeared in the American literature, a few notes will be appended and the plant described on a following page.

In size and shape the species corresponds most closely to *P. chioneus,* but it is of a different color and the spores are ellipsoid to subglobose. From *P. galactinus* and *P. caesius* it is separated by the almost or quite glabrous pileus and from the latter also by the spores. The writer finds it most often on dead willow logs in willow thickets along river bottoms. The types were described from similar locations. Fresh plants have the same peculiar fragrant odor that is found in *P. galactinus.*

The following key will aid in the determination of the species here discussed:

Spores cylindric-oblong, often allantoid 1
Spores ellipsoid to globose 3
1. Pileus villous-strigose; hymenium often bluish or grayish blue..*5. P. caesius*
   Pileus glabrous or very slightly pubescent 2
2. Hyphae of context simple or very slightly branched; pileus usually triangular in section; tubes usually 4–9 mm. long .....*2. P. albellus*
   Hyphae of context much branched; pileus usually more applanate; tubes 1–3 mm. long .....*1. P. chioneus*
3. Spores 5–6 μ in longest direction; plants not fragrant when fresh 4
   Spores 2–4 μ in longest direction; plants fragrant when fresh 5
4. Pileus strigose-tomentose or strigose-hispid, especially on the margin; tubes collapsing on drying, the mouths equal, small, averaging 3–4 to a mm. .....*3. P. spumeus*
   Pileus glabrous or floccose-tomentose; tubes scarcely collapsing on drying, the mouths usually somewhat sinuous, averaging 1–2 to a mm. .....*4. P. delectans*
5. Pileus glabrous or nearly so .....*7. P. fumidiceps*
   Pileus conspicuously pubescent, often strigose-tomentose at the base .....*6. P. galactinus*

1. Polyporus chioneus Fries. Plate 24, fig. 13, 16b

Pileus soft and watery when fresh, rigid when dry, 2–7 × 1–6 × *0.5–1.5* cm., white, often grayish or yellowish when dry, glabrous or nearly so, *covered with a thin continuous gray or yellowish pellicle that becomes more evident when the plants are dried;* context white, *usually with a fragrant*

[1] Ann. Myc. 6: p. 61. 1908.

odor when fresh, soft and friable when dry, *2–7 mm. thick; tubes 1.5–3 mm. long,* the mouths white or yellowish, averaging 3–4 to a mm.; spores cylindric or allantoid, minute, hyaline, 3–4 × 0.7–1.5 μ; cystidia none; hyphae of context hyaline, much branched.

On dead wood of deciduous trees.

Specimens examined: Mo. Bot. Herb. 4311 (Missouri).—Burt Herb. (collections from Vermont and New York).—Overholts Herb. 2325, 2261, 2277, 2276 (New York), 2326 (Ohio).

2. **Polyporus albellus** Peck.

Plate 23, fig. 5, Plate 24, fig. 16a.

Pileus soft and watery when fresh, rigid when dry, *more or less triangular in section,* 1–8 × 1–7 × *1–4* cm., white or yellowish, glabrous or nearly so, *covered with a thin yellowish pellicle that is more evident in dried plants,* but often disappears in patches; context white, soft and friable when dry, *0.5–3 cm. thick;* tubes 4–9 mm. long, the mouths white or yellowish, averaging 3–4 to a mm.; spores cylindric or allantoid, minute, hyaline, 3–4 × 0.7–1.5 μ; cystidia none; hyphae of context hyaline, unbranched or nearly so.

On dead wood of deciduous trees.

Specimens examined: Mo. Bot. Gard. Herb. 43756 (Idaho).—Burt Herb. (collection from Pennsylvania).—Overholts Herb. 591 (Vermont), 408, 149, 207 (Ohio), 2243, 2270 (New York), 440 (Missouri).

3. **Polyporus spumeus** Sow. ex Hornemann.

Plate 24, figs. 10, 11, 14a.

Pileus soft and watery when fresh, rigid on drying, *5–20* × *6–20* × *2–6 cm.* (much thinner when dried), white or somewhat yellowish, *villous-strigose or matted strigose-tomentose;* context white, rigid on drying, 1–3 cm. thick; *tubes 0.5–1.5 cm. long,* collapsing when dried, the mouths white or yellowish, *averaging 2–4 to a mm.;* spores *ellipsoid to subglobose,* hyaline, smooth, often once guttulate, *5–6* × *4–5* μ; cystidia none.

Illustrations: Hornemann, in Fl. Dan. *pl. 1794.*—Berk. Outl. Brit. Fung. *pl. 16, f. 4.*

Specimens examined: Cooke, Fung. Brit. 511[1].—Thuem. Myc. Univ. 709[1].—Mo. Bot. Gard. Herb. 43719 (Missouri).—Overholts Herb. 101 (Ohio), 526, 625 (Missouri).

4. Polyporus delectans Peck. Plate 24, fig. 14b.

Pileus soft and watery when fresh, *3–15 × 5–20 × 1.5–5* cm., white, yellowish, or grayish, *glabrous to finely tomentose;* context white, often with a soft upper layer and a more firm lower layer, firm when dry, 0.5–2 cm. thick; tubes 0.5–1.5 cm. long, the mouths white or yellowish, *averaging 1–2 to a mm.; spores ellipsoid to subglobose,* often uninucleate, hyaline, smooth, *4–5 × 5–6 μ*; cystidia none.

Growing from wounds of living trees and on old logs.

Illustrations: Jour. Cinc. Soc. Nat. Hist. 8: *pl. 1.*

Specimens examined: Overholts Herb. 145, 519, 250, 415, 659, 93, 258, 255 (all from Ohio and Missouri).

5. Polyporus caesius Schrad. ex Fries.

Pileus more or less triangular in outline, rather soft and watery when fresh, 1–5 × 1–4 × 0.5–2 cm., white or grayish, *rarely bluish gray, villous-pubescent or strigose;* context white, 3–10 mm. thick; tubes 3–5 mm. long, white or *grayish blue, large, unequal, averaging 1–3 to a mm., the dissepiments thin, torn and lacerated; spores cylindric or allantoid,* smooth, hyaline, *3–4 × 0.7–1.5 μ*; cystidia none.

On dead wood of deciduous trees.

Illustrations: Sow. Col. Fig. Eng. Fung. *pl. 226* (as *Boletus albidus*).—Gill. Champ. Fr. *pl. 458.*

Specimens examined: Krieg. Fung. Sax. 1913.—Mo. Bot. Gard. Herb. 43650 (Missouri).—Burt Herb. (collections from Canada and New York).—Overholts Herb. 627 (Missouri), 2271 (New York).

[1] These specimens or sections of specimens are not well preserved. They contain no spores, and while the general appearance, i. e., shape of pileus, size of pores, length of tubes in comparison with thickness of context, etc., are very much the same, the context appears to be more woody and zonate than in our specimens. Ellis N. Am. Fung. 1103 is referred to *P. spumeus* Fries. It is the same as distributed by Cooke, Fung. Brit. 603, under the name *P. spumosus* Fries. There is no such species listed by Saccardo. Lloyd (Letter No. 52, p. 25) refers the Ellis specimen to *Fomes geotropus* Cooke.

6. Polyporus galactinus Berk.

Plate 24, figs. 12, 15, 17.

Pileus more or less triangular in sections, sometimes gibbous behind, rather firm but watery, 3–8 × 5–10 × 1–3 cm., white or yellowish, *strigose-tomentose at the base, short tomentose on the margin;* context fibrous when fresh, hard and *sometimes resinous when dry,* white, 0.3–2 cm. thick, *strongly zonate, with a strong fragrant odor in fresh specimens;* tubes 5–10 mm. long, the mouths white or yellowish, *minute, averaging 4–6 to a mm.;* spores *ellipsoid,* smooth, hyaline, once guttulate, minute, *3–4 × 2–3 μ;* cystidia none.

On old logs in woods, especially in overflow river bottoms.

Specimens examined: Mo. Bot. Gard. Herb. 4092, 43636 (Missouri), 4138.—Overholts Herb. 42, 489, 382, 134, 252, 2178, 511, 611, 583 (mostly from Ohio and Missouri).

7. Polyporus fumidiceps Atkinson. Plate 23, fig. 6.

Pileus *thin,* soft and watery when fresh, 1–4 ×2–5 × *0.5–1* cm., vinaceous buff to avellaneous or wood-brown, *minutely pubescent or glabrous;* context white, watery, *with a strong fragrant odor, 2–5 mm. thick;* tubes 2–5 mm. long, sometimes olive-green within on drying, the mouths concolorous, *averaging 4–5 to a mm.;* spores *ellipsoid to subglobose,* smooth, hyaline, *2.5–3.5 × 1.5–2.5 μ;* cystidia none.

On dead wood of deciduous trees, especially willows, in woods and along overflow river bottoms.

Specimens examined: Mo. Bot. Gard. Herb. 43712 (Missouri).—Burt Herb. (part of type collection, from New York).—Overholts Herb. 552, 2305, 2318 (Missouri).

Polyporus lucidus Leyss. ex Fries, P. Tsugae Murr., P. Curtisii Berk., and Closely Related Species

These species form a rather natural group of plants possessing the common character of a laccate or varnished pileus. *P. lucidus* was described in 1780 by Leysser (as *Boletus*) from plants collected in England. The description calls for a plant with a lateral stipe and it is so figured by English mycolo-

gists. *P. Curtisii* was described by Berkeley, in 1849,[1] from plants collected in South Carolina by Curtis. *P. Tsugae* was more recently described by Murrill[2] from plants collected in New York City on decaying trunks and stumps of *Tsuga canadensis*. *Ganoderma sessile* was described at the same time and by the same author.

In Murrill's first treatment of this section[3] *Polyporus lucidus* was reported as a synonym for *P. pseudoboletus*, the latter name being used for the plant. The species was reported as occurring in most of the states east of the Mississippi River with the exception of the New England states. *P. Curtisii* was there listed as a synonym for *P. pseudoboletus* with the remark that specimens referred to *P. Curtisii* were only variations of the other species, due to age, rapidity of growth, and perhaps to differences in the host. The next species described was *Ganoderma sessile* and that was described as differing from *G. pseudoboletus* in being annual and sessile, with a very acute margin and a more rugose surface. It was reported as occurring in Indiana, New York, Ohio, Alabama, Louisiana, and Kentucky. In the 'North American Flora,'[4] six years later, the names *Ganoderma pseudoboletus* and *Polyporus lucidus* were both entirely omitted and *P. Curtisii* was restored as a specific name. No comment was made as to why this was done, nor as to what disposition was made of the numerous collections previously referred to *Ganoderma pseudoboletus*. The writer has seen material referred to *G. sessile* by Murrill, and the supposition is that all collections, except those belonging under *Polyporus Curtisii*, were referred to his new species *Ganoderma sessile*. This supposition is borne out by the fact that the description of that species is there so amended as to include stipitate forms also, while the species as originally described was limited to sessile forms. We must also conclude that *G. sessile* was regarded by its author as distinct from *Polyporus lucidus* of Europe, else that name or an older one would have

[1] Lond. Jour. Bot. and Kew Gard. Misc. 1: p. 101. 1849.
[2] Bul. Tor. Bot. Club 29: p. 601. 1902.
[3] *loc. cit.*
[4] N. Am. Fl. 9: p. 120. 1908.

been used. Mr. Murrill remarks concerning *Ganoderma sessile*[1]: "Very similar in its stipitate forms to *Polyporus lucidus* of Europe." The American plants are usually referred to *P. lucidus* by European mycologists, and taking into account the general agreement with the European descriptions and illustrations, and the fact that Murrill has consistently failed to cite any distinguishing characters upon which the legitimacy of his species might be established, we must conclude that there is no such distinction to be made between the European and the American plants. The American plant is variable in respect to the presence or absence of a stipe, and that cannot enter into the discussion.

There is a tendency among mycologists[2] to disregard the *Ganoderma Tsugae* described by Murrill. To the writer this species appears to be a perfectly good one, although it cannot be differentiated on host character alone. A further discussion of this species is reserved for a following paragraph.

In 1908 Atkinson[3] described a species of *Ganoderma* which he called *G. subperforatum*. After an examination of the type specimens the writer referred[4] this species to *Polyporus lucidus*. This leaves us three species of this section of *Polyporus* that are found in the central states. There are no spore characters of sufficient importance or constancy that can be used in separating them. There is a color difference but it probably cannot always be relied upon. The pileus of *P. Tsugae* is shining and mahogany-colored or darker; that of *P. lucidus* is of a lighter red color; and that of *P. Curtisii* is yellowish, at least in mature plants. Moreover, *P. Curtisii* is southern in its distribution, not being found north of the Ohio River; *P. Tsugae* is not reported south of Virginia; and *P. lucidus* is not limited in its north and south distribution in the United States.

[1] Northern Polypores, p. 55. 1914.

[2] cf. Atkinson, Bot. Gaz. 46: p. 335. 1908. *G. Tsugae* is here listed as a synonym for *G. pseudoboletus* (=*P. lucidus*). Later on the same page it is given varietal rank; also Lloyd (Letter No. 52, p. 27) cites it as a synonym for *Fomes lucidus*.

[3] Bot. Gaz. 46: p. 337. 1908.

[4] *loc. cit.* p. 123.

A more constant difference that serves to separate *P. Tsugae* is the color of the context. In *P. lucidus* and *P. Curtisii* the context is never pure white, but is usually separated into an upper light-colored and a lower brown layer. This lower layer is more firm than the upper one and often contains horny fibers. In *P. Tsugae* the context is uniform in texture and almost pure white throughout, but often with a very slight tinge of brown next the tubes. Under the microscope this effect is magnified. There are no brown hyphae in the context of *P. Tsugae*, while in the other two species brown hyphae are very pronounced, especially in the layer of context next the tubes. A comparison of the size of the hyphae in the three species is interesting but does not always give conclusive evidence as to the identity of the species. The hyphae of *P. Curtisii* vary from 4 to 6 μ in diameter. Those of *P. lucidus* are more variable. In some cases they cannot be differentiated from those of *P. Curtisii* in point of size, but in some specimens they attain a diameter of 10 μ. Those of *P. Tsugae* often attain a diameter of 15 μ. The difference in the branching of the hyphae of these three species is very striking and is shown in figs. 5 and 6, all drawn to the same scale. Figure 5a represents the hyphae of *P. Curtisii*, which are not extremely branched but can by no means be said to be unbranched. Figure 5b shows the hyphae of *P. lucidus*, and the branching does not differ materially from that of *P. Curtisii*. In both species the large hyphae may extend more than across the field of the high-power microscope and not branch at all in that distance. This condition is never found in the hyphae of *P. Tsugae*. There the hyphae are extremely branched, as shown in fig. 6. The large hyaline

Fig. 5. *a*, hyphae of *P. Curtisii*; *b*, hyphae of *P. lucidus*; *c*, width of hyphae of *P. Tsugae*.

hyphae are not continuous for any distance but break up into numerous smaller branches that are often rapidly narrowed to fine thread-like hyphae. This condition must be seen to be best appreciated. It affords, however, another character on which the species can be separated from those closely allied.

The following brief diagnoses of these species is appended:

1. Polyporus Curtisii Berk.

Plants perhaps always stipitate; pileus reniform or flabelliform, 3–12 × 3–20 × 0.7–2 cm., covered with a thin crust that is *at least in part ochraceous in mature plants,* zonate; context *soft and nearly white above, brown and firmer next the tubes,* 0.5–1.5 cm. thick; tubes 0.3–1.2 cm. long, the mouths white to brownish, averaging 3–5 to a mm.; stipe lateral, with color and context as in the pileus; spores light brown, ovoid with a truncate base, apparently echinulate, 8.5–11.5 × 4.5–7 μ; cystidia none; hyphae of context hyaline or brown, *4–6 μ in diameter.*

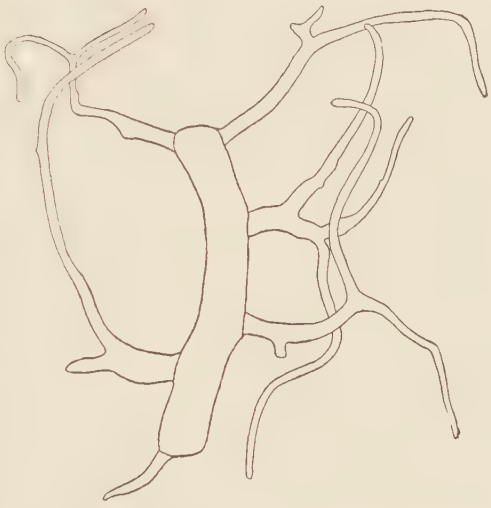


Fig. 6. Hyphae of *P. Tsugae.*

On and about trunks of *deciduous* trees.

Illustrations: Bot. Gaz. **46**: *f. 1–3.*

Specimens examined: Ell. N. Am. Fung. 802.—Rab.-Wint. Fung. Eur. 3430.—Mo. Bot. Gard. Herb. 1438 (Louisiana), 4746 (Alabama).—Overholts Herb. 305 (Florida), 962, 518 (Missouri), 2235 (New York). Also reported from most of the other states east of the Mississippi and south of the Ohio Rivers.

2. Polyporus lucidus Leyss. ex Fries.

Plants sessile or stipitate; pileus dimidiate or reniform, 3–12 × 3.5–20 × 0.5–2.5 cm., covered with a thin *reddish or*

chestnut crust, zonate; context white to light brown, *usually separated into an upper light-colored layer and a lower brown layer, never entirely white,* 0.2–1.5 cm. thick; tubes 0.3–1.5 cm. long, the mouths white to umber, averaging 3–5 to a mm.; stipe lateral or excentric when present, with color and context as in the pileus; spores light brown, ovoid with a truncate base, smooth or appearing echinulate, 9.5–11 × 5–6.5 μ; cystidia none; hyphae of context hyaline or brown, branched, *4–10 μ in diameter.*

On and about stumps and trunks of *deciduous* trees.

Illustrations: Bot. Gaz. **46**: *f. 5.*—Dufour, Atlas Champ. *pl. 49. f. 116.*—Gill. Champ. Fr. *pl. 457.*—Hard, Mushrooms, *f. 332.*—Krombh. Abbild. u. Beschr. *pl. 4. f. 22–24.*—Rostk. in Sturm's Deutsch. Fl. **3**: fasc. 5. *pl. 13.*

Specimens examined: Ell. N. Am. Fung. 5.—Ell. & Ev. Fung. Col. 202 (Delaware).—Krieg. Fung. Sax. 1116.—Rav. Fung. Am. 5.—Thuem. Myc. Univ. 104.—Mo. Bot. Gard. Herb. 43149, 4095, 4024, 4144 (Missouri), 43939 (Illinois).—Burt Herb. (collection from Vermont).—Overholts Herb. (collections from New York, Florida, Ohio, Illinois, and Missouri).

3. Polyporus Tsugae Murrill ex Overholts n. comb.

Plants stipitate; pileus flabelliform or reniform, 5–15 × 7–20 × 1–4 cm., with a *mahogany-colored or almost black, shining,* incrusted surface, sulcate; context *white or nearly so throughout,* 0.5–2 cm. thick; tubes 0.5–1 cm. long, the mouths white to brown, averaging 4–6 to a mm.; stipe present, with color and context as in the pileus; spores light brown, ovoid with a truncate base, apparently echinulate, 9–11 × 6–7 μ; cystidia none; *hyphae of context very irregular and much branched, up to 15 μ in diameter.*

On or about stumps and trunks of *hemlock and pine.*

Specimens examined: Burt Herb. (collection from Vermont).—Overholts Herb. 2338 (Vermont).

Fomes Ellisianus And. and F. fraxinophilus Peck

Fomes fraxinophilus was described by Peck from New York in 1882. It was first described as a *Polyporus* and later trans-

ferred to the genus *Fomes*. *F. Ellisianus* was described from Montana by Anderson in 1891, and redescribed as *Polyporus circumstans* by Morgan from South Dakota in 1895. The former species is abundant in the central and eastern United States, growing only on the trunks of ash trees. The latter species is found occasionally in the western United States, growing only on trunks of *Shepherdia*.

Lloyd has recently expressed the opinion that these two species are identical, except for host, and he has so treated them in his recent synopsis of the genus *Fomes*. The plants are much alike in their old stages but I cannot agree with him that *Fomes Ellisianus* is "*exactly the same plant*" as our eastern species on the ash. First, there is the distinction in host, but that of itself would not be important. Second, plants of *F. Ellisianus* that are fairly mature have a decidedly corrugated or radiate-rugose surface and a reddish tinge of color. I have seen no indication of either of these characters in *F. fraxinophilus* though I have been familiar with that species for a number of years and have observed it in all stages of growth. When the plants are several years old they become similar in appearance and it would be an easy matter to mistake the one for the other if the host were unknown. But the characters pointed out here are believed to be amply sufficient for retaining the two plants as distinct species.

The following brief descriptions are appended:

1. Fomes Ellisianus Anderson.

Pileus convex to ungulate, 3–10 × 3–8 × 1.5–4 cm., pallid to brown, *radiate-rugose and with a reddish tinge when young, black and usually somewhat rimose with age,* sulcate; context pallid to wood-colored, punky to corky, 0.5–2 cm. thick; tubes 2–6 mm. long each season,[1] *not distinctly stratified,* the mouths white or yellowish, *averaging 2–3 per mm.;* spores oblong-ellipsoid to broadly ellipsoid, 6–8 × 4–5 μ; cystidia none; hyphae hyaline, 3–5 μ.

On Shepherdia in the west-central states.

[1] The tubes in this plant are sometimes continuous to a length of 1.5 cm., but I do not believe that such lengths are attained in a single year's growth.

Illustrations: Bot. Gaz. **16**: *pl. 12.*—Jour. Cinc. Soc. Nat. Hist. **18**: *pl. 1. f. 4* (as *P. circumstans* Morg.).

Specimens examined: Anderson, Paras. Fung. Mont. 537 (as *P. fraxinophilus*).—Baker, Pl. N. N. Mex. 55.—Mo. Bot. Gard. Herb. 4272 (New Mexico).—Burt Herb. (collections from Montana and New Mexico). Also reported from North Dakota and Colorado.

2. Fomes fraxinophilus Peck.

Pileus convex to somewhat ungulate, 2–25 × 3.5–40 × 1.5–10 cm., *at first white,* soon grayish black or black, *not rugose, somewhat rimose* with age, sometimes sulcate; context woody, 0.5–1.5 cm. thick; tubes 2–4 mm. long each season, *indistinctly stratified,* the mouths white to brownish, *averaging 2–3 to a mm.;* spores ellipsoid to ovoid, 5–6 × 6–7 μ; cystidia none; hyphae 3–5 μ.

On living or dead ash trees.

Illustrations: U. S. Dept. Agr., Bur. Pl. Ind. Bul. 32: *pl. 2.*—Hard, Mushrooms, *f. 350.*

Specimens examined: Ell. & Ev. N. Am. Fung. 3302 (Kansas); Fung. Col. 909 (Kansas).—Mo. Bot. Gard. Herb. 4780, 1437, 4826 (Missouri).—Burt Herb. (collections from Kansas).—Overholts Herb. 46, 157, 159, 122, etc. (Ohio), 559, 624 (Missouri), 626 (Iowa). Also reported from Kentucky, Nebraska, Pennsylvania, Indiana, and New York.

Fomes igniarius Linn. ex Gillet and F. nigricans Fries

Much confusion has existed concerning the limits of these two species, and many different ideas are stated in the literature. Murrill has referred *Fomes nigricans* as a synonym for *F. igniarius.* Lloyd has kept them apart, though recognizing a close relationship between them. Others have concluded with Bresadola that we are here dealing with two species that can be easily separated on the presence or absence of setae in the hymenium. Romell has held that such is not the case, but that setae may be present or rare in either species, and has stated that they are usually most abundant near the bottom of the tubes. This would account for the fact that some

observers have stated that they have been unable to find setae in the hymenium of *F. nigricans.*

The original illustration of *F. nigricans* does not agree with any present-day conception of what the species really was. The manner in which the plates for Fries' 'Icones' were gotten together does not at all preclude the existence of grave errors regarding the identity of the species there illustrated. Hence the original illustration of *F. nigricans* has been discounted by careful European workers, they preferring to base the species rather on specimens authenticated by Fries himself. Of these, there appear to be specimens both at Upsala and at Kew.

The *F. nigricans* of my 'Ohio Polyporaceae' proves to be *F. Bakeri* Murrill. The specimens referred by me to *F. igniarius* are of two types. One of these has the pileus convex or ungulate, the surface sometimes becoming rimose, and setae not at all abundant. The second type is most commonly found on birch trees. The pileus is plane or slightly convex, sometimes shining black in color, and the surface often cracks in both directions but does not become roughly rimose. The setae are often more abundant. Of this second form, Lloyd recently wrote as follows concerning a collection sent to him by me: "It agrees with his (Fries') specimens (of *F. nigricans*) both at Upsala and at Kew. . . . . It is usually thinner than typical *F. igniarius* and the setae are more abundant than in the type form."

On the strength of this information I am now able to separate my collections of these forms into what I am convinced are the two species, *F. igniarius* and *F. nigricans,* respectively. I have examined all available material of the two species and have thoroughly confirmed Romell's observation on the presence of the setae. In but one collection was I unable to find setae and I do not doubt that further attempts would show their presence in that instance. It is advisable, however, as stated on a previous page of this article, to cut *longitudinal* sections of the hymenium, since by so doing one will be more likely to strike the setae if there is any variation in their abundance at particular places in the tubes.

The characters cited above do not appear to the writer to be sufficient to warrant the complete separation of the two species. They are sufficiently distinct, however, to enable one to refer to one form or the other all the specimens collected. It has been thought best to refer *F. nigricans* as a variety of *F. igniarius.*

The following diagnosis of the species and its variety is appended:

1. **Fomes igniarius** Linn. ex Fries.

Typical form: Pileus *convex or ungulate,* 3–10 × 5–20 × 2–10 cm., grayish black or black, *rarely roughly rimose with age,* not incrusted; context hard and woody, brown, 0.5–1 cm. thick; tubes 2–5 mm. long each season, the older layers conspicuously white-stuffed or incrusted, the mouths brown, averaging 4–5 per mm.; spores globose or subglobose, smooth, hyaline, 4–6 μ; setae present though sometimes rare, sharp-pointed, 16–25 × 6–8 μ; hyphae 3–4 μ.

Var. *nigricans* Fries: Pileus *plane to convex,* 3–10 × 3–15 × 2–7 cm., black, *sometimes shining black, the surface often cracked in both directions but never roughly rimose;* context and tubes as in the typical form, decidedly white incrusted; spores, setae, and hyphae as above, the setae often abundant.

On trunks of living deciduous trees.

Illustrations: Published illustrations passing under the name of this species and its variety are abundant, but typical representations of my plants so referred are scarce. The type form intergrades into the variety to such an extent that some illustrations are hard to refer. The typical form is represented by Hard, Mushrooms, *f. 349,* and in *pl. 25. f. 18.* of this paper. The variety is well represented by Lloyd, Myc. Notes 29: *f. 193;* Rostkovius in Sturm's Deutsch. Fl. 3: fasc. 17. *pl. 51.*

Specimens examined[1]: Ell. & Ev. N. Am. Fung. 915 (Kentucky).—Krieg. Fung. Sax. 526.—Thuem. Myc. Univ. 105.—Mo. Bot. Gard. Herb. 4037* (New York), 4043* (New York),

[1] Collections assigned to var. *nigricans* are marked with an asterisk.

43627* (Vermont), 42958 (Florida).—Burt Herb. (collections from Vermont and Canada).—Overholts Herb. 378* (Indiana), 423 (Ohio), 2460* (Vermont), 2256 (New York), 450 (Missouri).

Fomes scutellatus Schw. ex Cooke and F. ohiensis Berk. ex Murrill

These two species are closely related and have on more than one occasion been treated as a single species. *Fomes scutellatus* was first collected by Schweinitz on dead *Syringa* in Pennsylvania. It has since been reported on a few other hosts, namely, alder, witch-hazel, and sweet-gum. *F. ohiensis* was originally described from Ohio by Berkeley and is a very common species in that state. It is especially abundant on dead limbs on the ground in woods in September and October. Quite frequently it grows on fence posts, pickets, and a variety of other structural timbers. Both species were formerly frequently referred to the genus *Trametes,* but it seems best to restrict that genus to annual forms only.

Fig. 7. Hyphae of *F. ohiensis.*

Besides the host distinction, other characters may be used to distinguish between the two species. In typical specimens of *F. scutellatus* the pileus is entirely black and attached dorsally to the under side of branches. *F. ohiensis* is rarely found so attached, and the whole plant is at first white, the upper or basal part of the pileus becoming blackish with age, as in many species of *Fomes,* but the margin remaining white, even in perennial forms. *F. scutellatus* is rarely ungulate in form, while old specimens of *F. ohiensis* become steep in front, much as in *F. fomentarius.*

The spores of *F. scutellatus* have never been recorded and Lloyd has recently stated[1] that he has failed to find them even in freshly collected material. Murrill records them as

[1] Syn. Fomes, p. 218. 1915.

"*smooth, hyaline,*" but that conclusion is reached only from inference. I find them to be cylindric, hyaline, smooth, 8–9 × 2.5–3.5 μ. They thus differ from those of *F. ohiensis,* which are ovoid with a truncate base, hyaline, smooth, 10–12 × 6–7 μ. It is apparent then that the spores of *F. ohiensis* are similar in shape to those found in all species recently segregated into the genus *Ganoderma,* while those of *F. scutellatus* point to an alliance of that species with the genus *Trametes,* they being typical trametoid spores.

Fig. 8. Hyphae of *F. scutellatus.*

It is only in rare cases that the branching of the hyphae of the context can be used as a distinguishing character. The hyphae of *F. scutellatus* are much branched, while those of *F. ohiensis* are practically simple. These differences are shown in figs. 7 and 8.

It is thus apparent that these closely related species are separated by rather wide differences, and their determination need no longer be considered difficult.

The following descriptions are appended:

1. Fomes scutellatus Schw. ex Cooke.

Pileus *convex,* sometimes attached by the vertex and circular in outline, 0.5–1.5 × 0.5–2 × 0.1–0.5 cm., *entirely dark brown or black,* at least when mature, slightly sulcate; context corky, about 2 mm. thick; tubes 1–2 mm. long, the mouths white or pallid, averaging 4–5 per mm., thick-walled; spores *cylindric, 8–9 × 2.5–3.5 μ;* cystidia none; hyphae hyaline to light brown, *much branched,* 2–4 μ; basidia 6–9 μ broad.

Usually growing on alder and witch-hazel.

Specimens examined: Ell. & Ev. N. Am. Fung. 1597 (Pennsylvania); Fung. Col. 1010 (Vermont).—Mo. Bot. Gard. Herb. 4469 (New Jersey).—Burt Herb. (collections from New York and Vermont).—Overholts Herb. 337 (Ohio), 2394 (Florida). Also reported from Maine, Delaware, and Alabama.

2. Fomes ohiensis Berk. ex Murrill.

Pileus *convex to ungulate,* sometimes attached by the vertex and circular in outline, 0.5–2.5 × 0.5–3 × 0.2–1 cm., *pure white, then black at the base, the margin remaining white,* often zonate or sulcate; context corky or woody, 1–3 mm. thick; tubes 1–4 mm. long, the mouths white, averaging 3–5 per mm., thick-walled; spores[1] *ovoid with a truncate base, 10–12 × 6–7 μ*; cystidia none; hyphae hyaline, *unbranched,* 3–4 μ; basidia 8–11 μ broad.

On dead wood and on structural timbers.

Specimens examined: Ell. N. Am. Fung. 923 (as *Trametes*) (Ohio).—Burt Herb. (collection from South America, ex Herb. Romell).—Overholts Herb. 38, 39, 131, and others (Ohio), 479 (Missouri), 503 (Illinois). Also reported from Kansas, Michigan, and New York.

Trametes Pini Thor. ex Fries, T. Abietis Karst., and T. piceinus Peck.

Trametes Pini dates from the year 1803, when it was described by Thore,[2] and again in the following year, 1804, by Broteri.[3] The typical form of the perennial plant is rather large, has a more or less ungulate pileus, and in age becomes blackish and rimose. At times, however, the first year's growth is thin and applanate and thus differs markedly in form from the typical plant. This condition was observed by Peck and the name *Polyporus* (later changed to *Trametes*) *piceinus* was proposed by him for the form that he collected on *Picea* about 1889.[4] Karsten had already[5] described the same plant in Europe, in 1882, as *Fomes Abietis,* and the two names have been used interchangeably in this country for several years. In 1889 Karsten[6] referred to his species as

[1] According to Murrill (N. Am. Flora 9: p. 96. 1908) the spores of the size and form given here are conidial, but they represent the only type of spore I have been able to find in the hymenium of this species.

[2] Chlor. Land. p. 487. 1803.

[3] Fl. Lusit 2: p. 468. 1804.

[4] Rept. N. Y. State Mus. 42: p. 121. 1889.

[5] Bidrag Finl. Nat. Folk. 37: p. 242. 1882.

[6] Finl. Basidav, p. 336. 1889.

Trametes Pini var. *Abietis,* and that name has also appeared in the American literature. The writer has not seen Karsten's types and his opinion as to the synonymy of the species of Peck and Karsten is based entirely on the use of the names in this country and on the fact that *T. Pini* var. *Abietis,* as distributed by Romell,[1] is certainly to be referred to Peck's species. In the case of *Polyporus piceinus* and *Trametes Pini,* however, the evidence is not so clear, and there are yet mycologists who distinguish between the two species.

Peck has stated[2] that the pileus of *T. piceinus* is persistently tomentose, while that of *T. Pini* is not tomentose, and on this ground and also in view of the fact that the former is thin and applanate while the latter is thick and ungulate, the two have been kept apart to some extent, though Murrill, in 1908, declared them to be not specifically distinct. During the summer of 1913 and again in 1914 the writer had the privilege of collecting in the almost unexplored (mycologically) region of the Rocky Mountains in central Colorado. Here the forests are principally composed of the lodge-pole pine (*Pinus Murrayana*) and the Engelmann spruce (*Picea Engelmannii*), the former genus being the typical host of *T. Pini* and the latter the same for *T. piceinus.* No extensive field observations had been previously reported as to the intermingling of these supposed species of fungi, and the opportunity was taken to procure some notes on the subject. In that region the species is more abundant on the spruce than on the pine, probably because the best spruce forests follow the courses of the streams, while the pine often represents the only tree growth on the mountain sides and in the higher parts of the mountain parks where the soil often contains a higher percentage of sand. Such forests are not dense and quickly become dry, unless kept moist by daily rainfalls. Hence the statement that *T. Pini* is more often found on spruce in that locality is not surprising. In one instance in an area of no more than four square feet on a spruce snag the writer counted 18 sporophores, and of these about half were the *T. Pini* form and

[1] Fung. Scand. 7.

[2] Rept. N. Y. State Mus. 54: p. 170. 1901.

the rest were good specimens of the thin form known as *T. piceinus*. There is no doubt in the writer's mind that all these sporophores came from a common mycelium. In 1914 a similar find was made, the substratum being an old spruce log. Portions of these two collections are preserved in the writer's herbarium. Attempts were later made to separate the specimens in these collections by means of microscopic characters, but it was found to be impossible. In view of these observations it is seen that the recently expressed opinion of Meinecke[1] that the variation in shape is due to the host, is not true for the fungus, as it sometimes occurs in Colorado.

In some localities it may be more convenient to consider the thin form as a variety of *T. Pini,* for it must be admitted that the two forms do not always grow in such close association as described above. Yet the evidence is clear that they cannot be regarded as distinct species.

The writer believes that it will add to the clearness of the general situation in the *Polyporaceae* to include in the genus *Fomes* all perennial plants of whatever structure. This not only simplifies the definition of the genus *Fomes,* but also gives a clearer idea of the genus *Trametes.* As it has been commonly understood, the genus *Trametes* is a very poorly defined one, and any attempt to make its limits clearer is a step in the right direction. The transfer of this species to *Fomes* has already been made by Lloyd[2]. The species is here described under that name.

1. Fomes Pini Thor. ex Lloyd.

Sporophores very variable, the variations grouping themselves as follows:

Typical form: Sporophore *perennial, often ungulate, 6–15* × *4–20* × *1–15* cm., at first tawny and with elevated zones of appressed tomentum, becoming blackish and glabrous, the surface cracking or becoming rough and irregular; context not more than 5 mm. thick, tawny or ochraceous tawny, woody; tubes 2–6 mm. long each season, the mouths ochraceous to

[1] Forest tree diseases common in California and Nevada, p. 43. 1914.

[2] Syn. Fomes, p. 275. 1915.

brown; spores globose or subglobose, hyaline, 4–5 μ broad; setae abundant, sharp-pointed, brown, extending 20–30 μ beyond the basidia; hyphae 3–5 μ.

Var. *Abietis* Karsten: Sporophores *usually annual, rather thin and applanate, 1–5 × 1–7 × 0.3–1* cm., tawny or russet-tawny toward the margin, the immediate margin sometimes brighter-colored, zonate with elevated ridges of tomentum, grayish black or brownish black toward the base; context colored as in the typical form, 1–3 mm. thick; tubes *usually in a single layer;* spores, setae, and hyphae, as in the typical form.

On wood of coniferous trees, both living and dead.

Illustrations: Boudier, Ic. Myc. *pl. 161.*—Delacroix, Atlas Path. Veget. *pl. 19. f. 10–12.*—Meinecke, For. Tree Dis. Calif. and Nev. *pl. 4–5.*—Rostk. in Sturm's Deutsch. Fl. 3: fasc. 17. *pl. 50.*

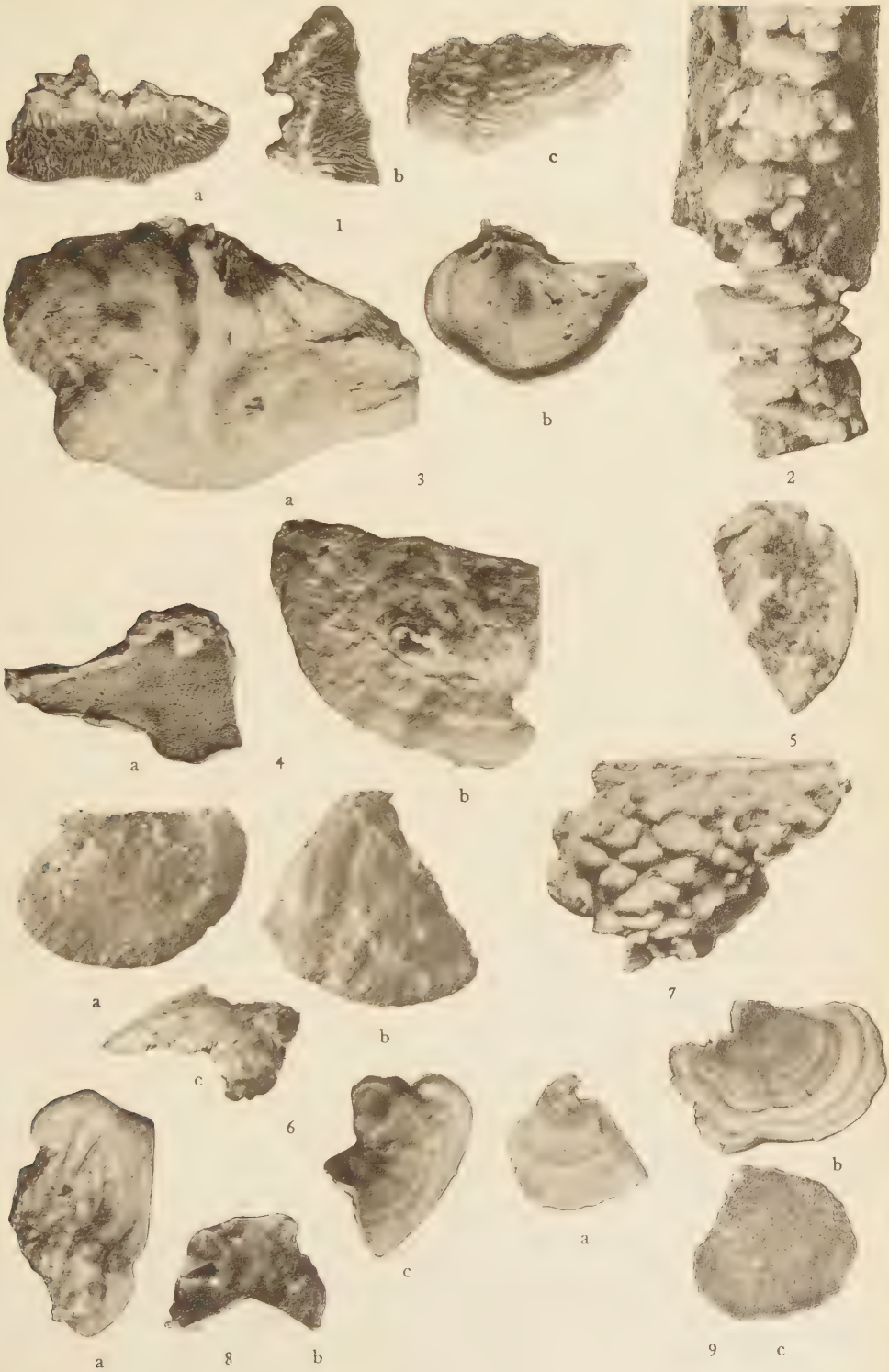
Specimens examined: Ell. N. Am. Fung. 602 (New Jersey).—Ell. & Ev. N. Am. Fung. 2507 (as *T. Abietis*) (Canada).—Linh. Fung. Hung. 348.—Rabenh. Crypt. Samm. Schule & Haus 8; Herb. Myc. 118.—Romell, Fung. Scand. 7 (as *T. Pini* var. *Abietis.*—Seym. & Earle, Econ. Fung. 11: 549.—Mo. Bot. Gard. Herb. 42958 (Washington), 4609 (Newfoundland), 42970 (Maine), 42954 (Michigan), 42956 (Vermont), 4618 (Colorado), 43810 (Missouri), and others.—Overholts Herb. 154 (Ohio), 630, 2033, 642, and 2391 (Colorado), 2458 (Montana), and others.

Graduate Laboratory, Missouri Botanical Garden.

Explanation of Plate

PLATE 23

Fig. 1. Specimens of *P. abietinus* with lamellate hymenium.
Fig. 2. Surface view of typical sporophores of *P. abietinus*.
Fig. 3. Typical sporophores of *P. fumosus*.
Fig. 4. *P. Burtii*. Photograph of type specimens.
Fig. 5. Upper surface of *P. albellus*.
Fig. 6. *P. fumidiceps*, showing upper surface and section through a sporophore.
Fig. 7. *P. crispus*, showing the densely imbricate mode of growth and the pubescent pileus.
Fig. 8. *P. adustus*. View of surface of pileus and hymenium.
Fig. 9. Typical sporophores of *P. pargamenus*.

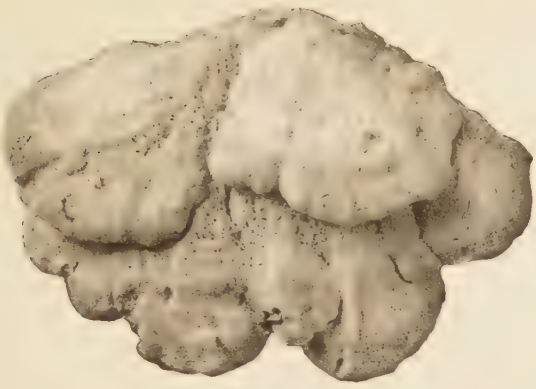


OVERHOLTS—POLYPORACEAE

Explanation of Plate

PLATE 24

Fig. 10. View of hymenium of *P. spumeus*.
Fig. 11. The pores of *P. spumeus* somewhat enlarged.
Fig. 12. Section through a sporophore of *P. galactinus*. Note the prominent zonation of the context.
Fig. 13. Upper surface of *P. chioneus*.
Fig. 14. Comparison of the size of the tubes in (a) *P. spumeus* and (b) *P. delectans*.
Fig. 15. Upper surface of *P. galactinus*. Note the prominent pubescence.
Fig. 16. Sections showing the relative thickness of the pilei in (a) *P. albellus* and (b) *P. chioneus*.
Fig. 17. Hymenium of *P. galactinus*.



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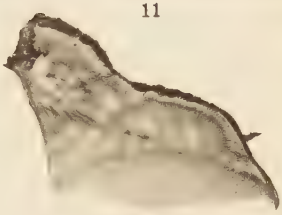

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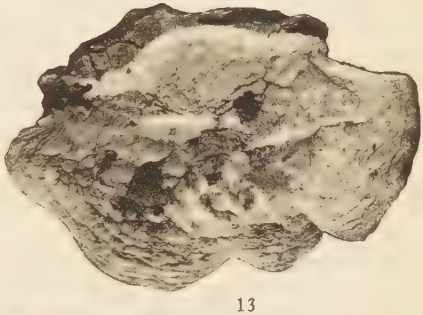

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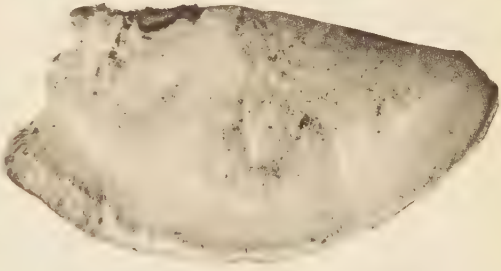

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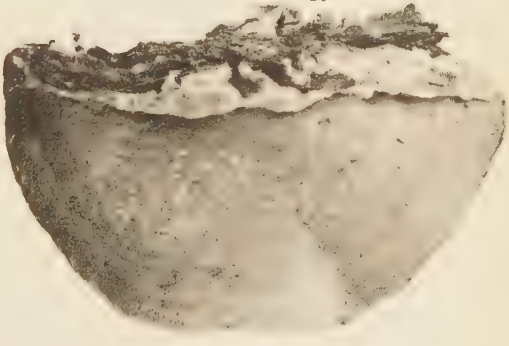

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OVERHOLTS—POLYPORACEAE

Explanation of Plate

PLATE 25

Fig. 18. Sporophore of *F. igniarius* growing on beech trunk.

Fig. 19. View of upper surface and section through a sporophore of *F. fraxinophilus.*

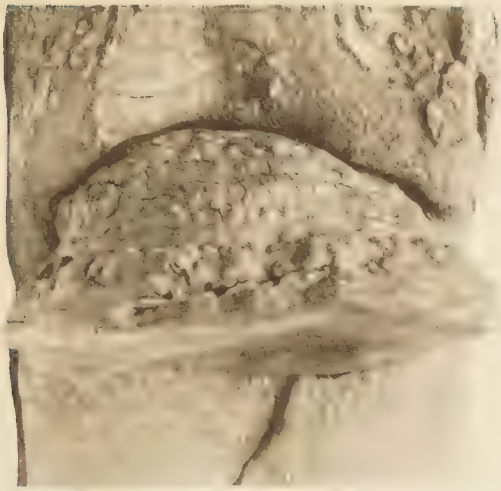
Fig. 20. Section through a typical sporophore of *F. igniarius* var. *nigricans.* Note the strongly white incrusted layers of tubes and context.

Fig. 21. Surface view of the same specimen of *F. igniarius* var. *nigricans.*

Fig. 22. Sporophores of *F. ohiensis.*

Fig. 23. *F. Ellisianus,* showing rugose upper surface and section of hymenium with long tubes.

Fig. 24. Sporophores of *F. scutellatus* on limbs of alder.



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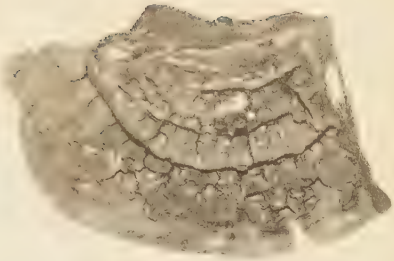

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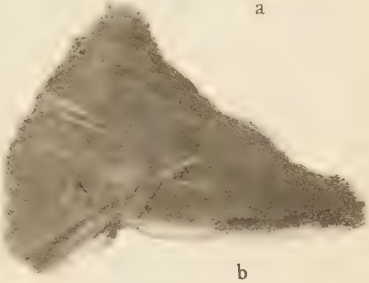

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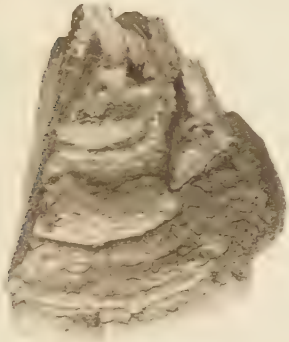

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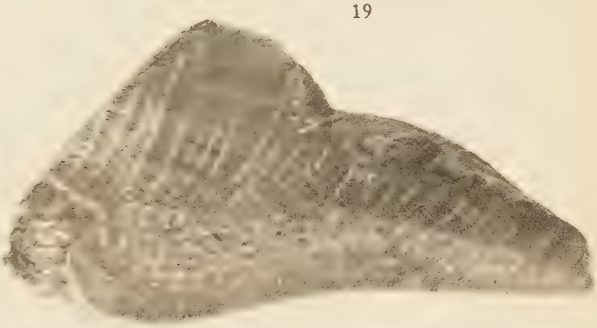

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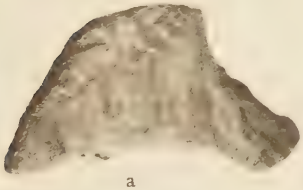

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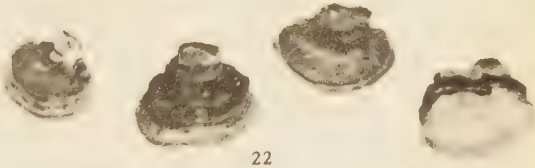

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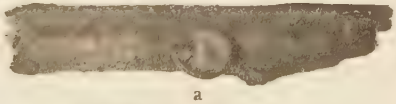

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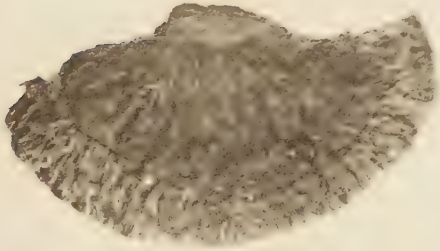

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OVERHOLTS—POLYPORACEAE

THE THELEPHORACEAE OF NORTH AMERICA V[1]

Tremellodendron, Eichleriella, and Sebacina

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The group of fungi comprising the present part probably attains its greatest development both in form and numbers in the western continent where it culminates in the erect *Tremellodendron,* apparently confined to North America. This continent has five of the seven species of *Eichleriella;* it has twenty-six species of *Sebacina* against fifteen for the Old World.

The better-known species of these genera were originally described in *Thelephora, Stereum,* and *Corticium,* with which they conform so closely in general habit of growth and consistency of the fructification that it is impossible to separate them from the latter except by microscopic examination of preparations which show the mature basidia to be longitudinally cruciately septate. Collectors invariably roughly grade their findings of *Sebacina* as *Corticium.* The recognition of longitudinally septate basidia is not always easy with the aid of the microscope; for example, the fungus originally described as *Stereum Leveillianum* B. & C. has been studied critically at several times by experts without their observing the true structure of the basidia.

I regret that the present account of our species and their range in North America does not include all the material at hand. The Missouri Botanical Garden herbarium contains several hundred undetermined specimens of possible Corticiums which have been received during the last two years.

Note.—Explanation in regard to the citation of specimens studied is given in Part I, Ann. Mo. Bot. Gard. 1 : 202. 1914, footnote. The technical color terms used in this work are those of Ridgway, Color Standards and Nomenclature. Washington, D. C., 1912.

[1] Issued December 20, 1915

I have looked through these collections very carefully to sort out, without examination now of everything by microscopic methods, just those specimens which ought to be studied at once for citation in this part, but some of the specimens most desirable for citation have undoubtedly been deferred for the present as probable Corticiums.

As it is really a nice microscopical task to recognize longitudinally septate basidia when they are not at their best, some notes, based on my experience, may be helpful. Species of *Tremellodendron* are the most easily recognized, for a little of the moistened and softened hymenium may be picked out with a scalpel, placed in a drop of water, stained with aqueous solution of eosin, 7 per cent potassium hydrate solution added, and then crushed down by pressure on the cover glass. In the detection of species of *Eichleriella* and *Sebacina*, thin vertical sections of the fructification are necessary. After the sections have been made turgid and clear by potassium hydrate solution, the latter should be drained off and the sections stained by merely a sufficient amount of solution of Gruebler's eosin soluble in alcohol, and mounted in water for temporary examination. It may be necessary to spread apart the tissues of the preparation somewhat by pressure upon the cover glass. If the preparation is to be preserved permanently in glycerin, a drop of dilute solution of sodium chloride should be run under the cover glass before the glycerin is added to insure a permanent stain by the Gruebler eosin.

Longitudinally cruciately septate basidia are simple and pyriform or subglobose when young, but so are the probasidia of *Septobasidium*, the possible storage organs of *Corticium polygonium*, and the basidia of some species of *Corticium*. The basidia of the latter are likely to form a layer at the surface of the fructification and are certainly simple if any can be detected bearing sterigmata and perhaps spores while still non-septate. In a fructification having longitudinally septate basidia, the hymenial surface is usually composed of paraphyses and of long, slender sterigmata arranged side by side; in this surface layer—but sometimes at a con-

siderable distance from the surface, as in *Thelephora Helvelloides* Schw.—is situated the layer of basidia. Only very rarely do the basidia of *Sebacina* or *Eichleriella* constitute the surface of the fructification.

If a fructification contains a palisade layer of deeply staining, pyriform bodies among or underneath the paraphyses and with no simple basidia in the surface layer, more or less prolonged examination of the pyriform bodies is likely to show longitudinal septa in some of them.

The three genera which comprise the present part of this monograph, are treated here by the writer, because their general habit and consistency conform so closely with *Thelephoraceae* having simple basidia, that they may be regarded as a connecting group, although belonging with the *Tremellaceae* by the structure of their basidia. Such of the species as were described in the past were described as *Thelephoraceae* or by authors with special knowledge of the *Thelephoraceae;* the taxonomic recognition of fungi of these genera seems likely to continue to fall in the future to students of the *Thelephoraceae,* for other mycologists will hardly care to glean for material of so few species among the many *Thelephoraceae* of similar aspect.

TREMELLODENDRON

Tremellodendron Atkinson, Jour. Myc. 8: 106. 1902; Saccardo, Syll. Fung. 17: 208. 1905.

The type species is *Merisma candidum* Schw.

Fructifications coriaceous, erect, pileate, branched or rarely simple; hymenium amphigenous or inferior; basidia longitudinally cruciately septate; spores white, even.

The species of *Tremellodendron* are indigenous to North America; none have been reported for other regions, so far as I am aware. The fructifications spring up on the ground in deep woods during wet weather in summer and early autumn, and have the general habit of *Thelephora vialis,* of branched Clavarias, or, very rarely, of simple clubs. In active vegetative condition the fructifications may be distinguished from species of *Clavaria* of similar habit by coriaceous and

tough consistency and by lack of brittleness. The longitudinally septate basidia afford a decisive character in all doubtful cases.

The specific distinctions between the more common species of this genus are based largely upon the form of mature and well-developed fructifications; very young, deformed, or fragmentary specimens can not be referred very confidently to their species.

Key to the Species

Fructifications branched when well developed. Simple forms may be present when very young or in the same colony with normal branched forms 1
Fructifications simple 4
1. Fructifications normally cespitose, more or less grown together..... 2
1. Fructifications solitary or scattered..... 3
2. With pileate divisions flattened, grown together at many points of contact, forming rosette-like masses 2–15 cm. in diameter..*1. T. pallidum*
2. With the stems grown together into a main stem 2–10 mm. thick; pileate divisions cylindric, spreading, grown together at only few points of contact; the smaller divisions about 1½ mm. thick..... .....*2. T. candidum*
2. Sometimes with both stems and pileate divisions grown together into compact bundles, usually merely closely cespitose and with the branches intricately intertangled; much slenderer than preceding species and with the habit of *Pterula*.....*5. T. merismatoides*
3. Stem about 1½ mm. thick, palmately few-branched; branches once or twice similarly branched, cylindric or subcylindric, often channelled on the upper side; basidia 15×9 μ; spores 9–15×4½–6 μ, pointed at the base only .....*3. T. Cladonia*
3. Stem about ½–1 mm. thick, sometimes with occasional, scattered, divergent branches from its side, dilated at the upper end, divided into a few, short, finger-shaped branches; basidia 20–24×12–14 μ; spores 14–16×6–7 μ, pointed at both ends, Known from Jamaica only.....*4. T. tenue*
4. Fructification dark orange, probably with medullary tissue pale as in all the preceding species; basidia subglobose, 10–12 μ in diameter .....*6. T. aurantium*
4. Fructification black with the exception of the hymenium; hymenium olive-ocher, amphigenous on the lower third of the fructification; basidia 11×7 μ. Known from Porto Rico only.....*7. T. simplex*

1. **Tremellodendron pallidum** (Schw.) Burt, n. comb.

Plate 26, fig. 6.

Thelephora (*Merisma*) *pallida* Schw. Am. Phil. Soc. Trans. N. S. **4**: 166. 1834.—*T. Schweinitzii* Peck, N. Y. State Mus. Rept. **29**: 67. 1878; Saccardo, Syll. Fung. **6**: 534. 1888.—*Tremellodendron Schweinitzii* (Peck) Atk. Jour. Myc. **8**: 106. 1902.

Illustrations: Hard, Mushrooms *f. 381.*—Moffatt, Chicago Acad. Sci. Bul. 7: *pl. 22. f. 1.* 1909.

Type: in Herb. Schweinitz and a portion in Curtis Herb.

Fructification cespitose, erect, white or pallid, drying warm buff, stipitate by one to several or many stems which may be distinct below or arise from a common, swollen, basal mass; above, the stems branch into flattened, more or less furrowed, pileate divisions which grow together at surfaces of contact to form a somewhat cup-shaped or rosette-like mass; divisions in center of mass somewhat subulate at the apex, those at margin dilated and sometimes fimbriate, splitting when dry into sharp fibers or spicules; hymenium inferior, warm buff, best developed towards the base of the pileate divisions; basidia pyriform, longitudinally cruciately septate, 12–15 × 9 μ; spores from a spore collection, white, simple, 10–12 × 4½–5½ μ, and 9–12 × 4½ μ from an herbarium specimen.

Fructifications 2–10 cm. high, 2–15 cm. broad.

On the ground in deep woods. Canada to South Carolina and westward to Missouri. June to October. Common.

Full-grown and well-developed specimens are rosette-like and resemble *Thelephora vialis* when viewed from above but may have the pileate mass supported by many stems; small specimens with only a single stem do occur. The large specimens are apparently due to the concrescence of many small fructifications. In the large specimens the pileate divisions on the outside of the mass become broader and more flattened than those in the interior. The flattened form of the divisions of the pileus and their growing together at numerous points of contact are characters separating *Tremellodendron pallidum* from *T. candidum*. The small specimens, distributed as *T. pallidum* in published exsiccati, are often so immature and fragmentary that they cannot be distinguished from *T. candidum*.

Forms of *T. pallidum* which have the tips of pileate divisions split into sharp fibers or spicules are the *Thelephora cristata* and *T. serrata* of Schweinitz, 'Syn. N. Am. Fungi,' Nos. 621 and 623.

Specimens examined:

Exsiccati: Ravenel, Fungi Car. II, 29; Ellis, N. Am. Fungi, 510; Ell. & Ev., Fungi Col., 1208; Shear, N. Y. Fungi, 50.

Canada, Ontario: London, *J. Dearness,* and also in Ell. & Ev., Fungi Col., 1208; Belleville, *J. Macoun, 174, 230* (both in Can. Geol. Surv. Herb.).

Maine: N. Parsonfield, *R. G. Leavitt.*

Vermont: near Burlington, *L. R. Jones,* two collections; Middlebury, *E. A. Burt,* two collections.

Massachusetts: *Sprague, 773* (in Curtis Herb. under the name *Thelephora vialis*); Brookline, *S. Davis.*

Connecticut: East Hartford, *C. C. Hanmer;* and also No. 1567 (in Hanmer Herb.).

New York: Alcove, *C. L. Shear,* N. Y. Fungi, 50; Floodwood, *E. A. Burt;* Taughannock, *H. H. Whetzel,* Cornell Univ. Herb., 13600; Buffalo, *G. W. Clinton* (in U. S. Dept. Agr. Herb.); Tarrytown, *H. von Schrenk* (in Mo. Bot. Gard. Herb., ±2800).

New Jersey: Laning (in Mo. Bot. Gard. Herb., 701330, 701331, 701333); Newfield, *J. B. Ellis* (in Mo. Bot. Gard. Herb., 5162), and also N. Am. Fungi, 510.

Pennsylvania: Bethlehem, *Schweinitz,* type (in Herb. Schweinitz, and a portion in Curtis Herb. and also the Nos. 621 and 623 of Schweinitz, 'Syn. N. Am. Fungi,' under the names *Thelephora cristata* and *T. serrata,* respectively); Trexlertown, *W. Herbst* (in Lloyd Herb.); Kittanning, *D. R. Sumstine.*

Delaware: Newark, *H. S. Jackson, B10.*

District of Columbia: Washington, *O. F. Cook, 2,* comm. by P. L. Ricker.

Virginia: Great Falls, *C. L. Shear, 1044.*

North Carolina: Blowing Rock, *G. F. Atkinson,* Cornell Univ. Herb., 10666, 10667, 10669, 10664 (of which the first two numbers and part of the third are in Cornell Univ. Herb. and part of the third and the last in Mo. Bot. Gard. Herb.).

South Carolina: Ravenel, Fungi Car. II, 29.

Ohio: *C. G. Lloyd, 2346* (in Lloyd Herb.); Loveland, *D. L. James* (in U. S. Dept. Agr. Herb.).

West Virginia: Eglon, *C. G. Lloyd, 02601.*

Kentucky: *S. M. Price* (in Mo. Bot. Gard. Herb., 5141, 5144, 701332, 712372); Mammoth Cave, *C. G. Lloyd, 1071.*

Illinois: *H. C. Beardslee* (in Lloyd Herb., 2175); Newton's Ferry, *E. T. & S. A. Harper, 441;* Riverside, *E. T. & S. A. Harper, 696.*

Wisconsin: Blanchardville, Univ. of Wis. Herb., 52; Madison, *E. T. & S. A. Harper, 881; C. J. Humphrey, 948* (in Mo. Bot. Gard. Herb., 44783).

Iowa: *T. J. Fitzpatrick* (in Lloyd Herb.).

Missouri: St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 701335, 701370, 701371); Cliff Cave, *J. B. S. Norton* (in Mo. Bot. Gard. Herb., 5126); Columbia, *B. M. Duggar, 140;* Creve Coeur, *Miss E. M. Briggs* (in Mo. Bot. Gard. Herb., 44756).

2. **T. candidum** Schw. ex Atkinson, Jour. Myc. **8**: 106. 1902. Plate 26, fig. 3.

Merisma candidum Schweinitz, Naturforsch. Ges. Leipzig Schrift. **1**: 110. 1822.—*Thelephora candida* Fries, Elenchus Fung. 168. 1828; Schweinitz, Am. Phil. Soc. Trans. N. S. **4**: 166. 1834.

Type: in Herb. Schweinitz, Acad. Nat. Sci. Phila.

Fructifications cespitose, erect, coriaceous-soft, white, drying warm buff, stipitate; stem thick, palmately branched, with branches spreading, branching, cylindric or subcylindric; hymenium inferior on the main branches, often amphigenous on secondary branches; basidia longitudinally septate, 10–12 × 7½–9 μ; spores colorless, simple, even, 7½–10 × 4½–5½ μ.

Fructifications 2½–5 cm. high, 2–5 cm. broad; stem 2–10 mm. thick; smaller pileate branches about 1½ mm. thick.

On ground in open woods. Vermont to North Carolina and westward to Missouri. July to September. Infrequent.

The type of *T. candidum* has the dimensions given above for recent collections. In the original description Schweinitz noted that fructifications may attain a breadth of 15 cm.; at that time he had not given specific recognition to the large and common *T. pallidum* and it may be that the large specimens to which he referred were of the latter species. *T. candidum* is closely related to *T. pallidum* but contrasts with the latter in having consolidation between adjacent fructifications

confined to the main stems from the base upward to about the region of branching; from here the branches spread so that they grow together only rarely; furthermore, the branches are distinctly cylindric or subcylindric. The spores average a little shorter than those of related species.

Specimens examined:

Vermont: Lake Dunmore, *E. A. Burt;* Newfane, *C. D. Howe.*

Massachusetts: Woods Hole, *G. T. Moore.*

New York: Alcove, *C. L. Shear, 1218;* Fishers Island, *C. C. Hanmer, 192, 193, 194* (all in Hanmer Herb.).

North Carolina: *Schweinitz,* type (in Herb. Schweinitz); Blowing Rock, *G. F. Atkinson,* Cornell Univ. Herb., 10662, 10668 (in Mo. Bot. Gard. Herb., 44775, 44776) and (in Cornell Univ. Herb., 10663).

Ohio: Granville, *H. L. Jones.*

Missouri: near St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 701336).

3. T. Cladonia (Schw.) Burt, n. comb. Plate 26, figs. 1, 2.

Merisma Cladonia Schweinitz, Naturforsch. Ges. Leipzig Schrift. **1**: 110. 1822.—*Thelephora Cladonia* Fries, Elenchus Fung. 168. 1828; Epicr. 537. 1836–1838; Schweinitz, Am. Phil. Soc. Trans. N. S. **4**: 166. 1834; Saccardo, Syll. Fung. **6**: 535. 1888.—*Thelephora gracilis* Peck, Torr. Bot. Club Bul. **25**: 371. 1898.

Type: in Herb. Schweinitz.

Fructifications solitary or gregarious, erect, coriaceous-soft, pallid, drying warm buff, sometimes with the older portions pale olive-gray, stipitate; stem cylindric, palmately branched into a few—often three—cylindric branches, each or some of which occasionally branch again in similar manner; branches arranged in a plane from flattened end of stem or branch or in a circle about the cylindric end of the stem which is then sometimes perforate and the branches often channelled; hymenium amphigenous, or inferior when the branch is channelled; basidia longitudinally septate, pyriform, 15×9 μ; spores colorless, simple, even, curved, $9–15 \times 4\frac{1}{2}–6$ μ.

Fructifications 2½–5 cm. high, 7 mm.–2 cm. broad; stem about 1½ mm. thick.

On ground in woods. Canada to Mississippi and westward to Missouri. August and September.

The fructification of this species is smaller than that of *T. candidum* and has but few branches, which are often arranged in a circle about the end of the stem so as to appear somewhat proliferous on the margin of an imperfect cup as in some species of the lichen, *Cladonia*—hence the specific name—or with the branches standing up side by side from the compressed apex of the main stem. Both forms of branching have been found so associated in the same collection as to preclude the possibility of regarding this difference as a basis for two species. The branches are so frequently in threes that "trifaria" was contemplated as a name for the species by one author.

Specimens examined:

Canada: *J. Macoun, 78.*

Vermont: Smugglers Notch, *L. R. Jones;* Middlebury, *E. A. Burt;* Brattleboro, *C. C. Frost* (in Univ. Vermont Herb.).

Massachusetts: *Sprague, 871* (in Curtis Herb., 5762).

New York: Hague, *C. H. Peck, 7;* Ithaca, *G. F. Atkinson,* Cornell Univ. Herb., 7708.

Pennsylvania: Trexlertown, *W. Herbst* (in Lloyd Herb.).

District of Columbia: Takoma Park, *P. L. Ricker, 822* (in Ricker Herb.).

North Carolina: *Schweinitz,* type (Herb. Schweinitz and a portion in Curtis Herb.); Blowing Rock, *G. F. Atkinson* (in Cornell Univ. Herb., 10665, 10008. A part of the latter number is in Mo. Bot. Gard. Herb., 44774).

Georgia: Tallulah Falls, *A. B. Seymour,* Farlow Herb., O, P, Q, R, U, W (in Mo. Bot. Gard. Herb., 44619, 44623–44625, 44628, 44630).

Alabama: *F. S. Earle, 13,* type of *Thelephora gracilis* (in Coll. N. Y. State).

Mississippi: Biloxi, *Mrs. F. S. Earle, 32A.*

Ohio: Cincinnati, *A. P. Morgan* (in Lloyd Herb., 32); Loveland, *D. L. James.*

West Virginia: Eglon, *C. G. Lloyd, 02634.*
Missouri: Creve Coeur, *E. A. Burt* (in Mo. Bot. Gard. Herb., 44755).

4. T. tenue Burt, n. sp. Plate 26, fig. 7.

Type: in Burt Herb. and in N. Y. Bot. Gard. Herb.

Fructifications scattered, erect, very slender, coriaceous-soft, drying warm buff, stipitate; stem equal, flexuous, drying somewhat twisted and flattened, becoming fibrillose, sometimes giving off two or three scattered, divergent, small branches, dilated above and divided in a few palmately arranged, finger-shaped branches; hymenium inferior on the dilated portion and branches; basidia longitudinally septate, 20–24 × 12–14 μ; spores colorless, simple, even, curved, pointed at both ends, 14–16 × 6–7 μ.

Fructifications 2–3½ cm. high, 3 mm. broad; stem 1½–2½ cm. long, about ½–1 mm. thick.

On the ground in wet mountainous region, altitude 3000–5200 ft. Jamaica. December and January.

This species is characterized by its long and slender stem, few branches, and the largest basidia and spores of any species of the genus. The spores differ from those of the other species in being pointed at the apex.

Specimens examined:
Jamaica: Chester Vale, *W. A. & E. L. Murrill,* N. Y. Bot. Gard., Fungi of Jamaica, 400, type; Cinchona, *W. A. & E. L. Murrill,* N. Y. Bot. Gard., Fungi of Jamaica, 614.

5. T. merismatoides (Schw.) Burt, n. comb. Plate 26, fig. 4.

Clavaria merismatoides Schweinitz, Am. Phil. Soc. Trans. N. S. **4**: 182. 1834.—*Merisma Schweinitzii* Leveille, Ann. Sci. Nat. Bot. IV. **5**: 157. 1846.—*Lachnocladium merismatoides* (Schw.) Morgan, Cincinnati Soc. Nat. Hist. Jour. **10**: 193. 1888.—*Pterula merismatoides* (Schw.) Saccardo, Syll. Fung. **6**: 742. 1888.—*Thelephora merismatoides* Lloyd, Letter No. 26: 2. 1909. Nomen nudum.—*Tremellodendron merismatoides* Lloyd, Letter No. 40: 2. 1912. Nomen nudum.—*Thelephora pteruloides* Berk. & Curt., Hooker's Jour. Bot. **1**: 238. 1849; Grevillea **1**: 148. 1873.

Type: In Herb. Schweinitz, Acad. Nat. Sci. Phila.

Fructifications erect, cespitose or fasciculate, and sometimes with stems grown together, coriaceous, branched, pallid, drying with stems warm buff and branches tawny; branches few, rather straight, filiform, angular-terete; branchlets many, dilated and fimbriate at the apex, then splitting into spreading branchlets; hymenium glabrous, amphigenous; basidia longitudinally septate, pyriform, 12–15 × 8–9 μ; spores in preparations from herbarium specimens hyaline, even, simple, 8–10 × 4½–5 μ.

Cluster of fructifications 2–5 cm. high, 2–3 cm. broad. Individual from cluster has stem 5–10 mm. long, ½–1 mm. thick; branches about ¼–⅓ mm. thick.

On the ground in open woods. Massachusetts and New York to South Carolina and westward to Missouri. June to August.

This is a small species with the habit of a *Pterula* but with coriaceous structure and longitudinally septate basidia. The fructifications of a cluster may have their stems distant from one another by spaces equal to the diameter of the stems, but the branches interlock above; in other cases the fructifications are crowded closely together and united throughout their whole length. *T. merismatoides* may be distinguished from the preceding species by the smaller diameter of the stems and branches and from all the following species by its cespitose to fasciculate habit.

The collection from West Virginia, distributed as *Thelephora pteruloides* in Ell. & Ev., 'N. Am. Fungi,' 3415 and 'Fungi Col.,' 1117, has the hymenium composed of basidia standing side by side in a distinct palisade layer and the basidia not longitudinally septate in my opinion.

Specimens examined:

Massachusetts: near Boston, *Murray,* comm. by Sprague, 250 (in Curtis Herb. under the name *Thelephora pteruloides* B. & C.); Woods Hole, *G. T. Moore, 58.*

New York: Ithaca, *G. F. Atkinson, 37;* Fishers Island, *C. C. Hanmer, 1478* (in Hanmer Herb.).

New Jersey: Haddonfield, *T. J. Collins* comm. by C. G. Lloyd.

Pennsylvania: Bethlehem, *Schweinitz,* type (in Herb.

Schweinitz); York County, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 44742); Kittanning, *D. G. Sumstine.*

South Carolina: *M. A. Curtis, 1745* (the type and cotype of *Thelephora pteruloides* in Kew Herb. and Curtis Herb. respectively).

Ohio: Cincinnati, *A. P. Morgan,* Lloyd Herb., 2589 (determined by Morgan as *Thelephora filamentosa*).

Wisconsin: Lake Geneva, *E. T. & S. A. Harper, 842.*

Missouri: Meramec Highlands, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 44743).

6. **T. aurantium** Atkinson, Ann. Myc. **6**: 59. 1908.

Type: in Cornell Univ. Herb. but cannot be found at present.

"Plants simple, slender, 1–3 cm. long, 2–3 mm. stout, dark orange, tough. Basidia subglobose, 10–12 μ, longitudinally divided; sterigmata 4, long, slender, flexuous. Spores oboval-subelliptical, granular, then with an oil drop, 7–10 $\times$ 5–6 μ, white, hyaline.—C. U. herb., No. 10684, ground, woods, along small stream crossing Boone Road, Blowing Rock, Blue Ridge Mts., N. C. G. F. Atkinson, Aug. 19–Sept. 22, 1901."

—Original description.

T. aurantium differs from the preceding species of *Tremellodendron* by its simple fructifications. I have seen no specimens referable here. Professor Atkinson had intended to make a negative from his type so that I could include a figure of the species, but, upon going to the envelopes labelled *T. aurantium,* he found that they contained—by error of a helper—*T. merismatoides* instead. The specimens of *T. aurantium* have not been found.

7. **T. simplex** Burt, n. sp. Plate 26, fig. 5.

Type: in Mo. Bot. Gard. Herb. and in Farlow Herb.

Fructifications scattered, erect or suberect, drying hard, brittle, somewhat longitudinally wrinkled and sometimes compressed, black above, olive-ocher with the hymenium towards the base; hymenium amphigenous on the lower third of the fructification, olive-ocher, hyaline under the microscope, with surface consisting of colorless clavate paraphyses 5 μ thick,

and with basidia and spores at base of the paraphyses; basidia longitudinally septate; 11 × 7 μ; spores colorless, even, 7½–9 × 5–6 μ.

Fructifications about 2 cm. long, about 2 mm. thick.

In cane field. Porto Rico.

T. simplex is noteworthy by the column composed of longitudinally arranged, black hyphae, which extends the whole length of fructification and constitutes the whole, upper, sterile two-thirds of the fructification and is clothed by the ochraceous hymenium on the lower third. The specimens are broken off at the base, hence I cannot be sure that a stem was not originally present, but if present it would doubtless have been included in the packet. The general habit is that of a small *Geoglossum* or cylindric *Xylaria.*

Specimens examined:

Porto Rico: *J. R. Johnston,* comm. by W. G. Farlow, type (in Mo. Bot. Gard. Herb., 5119).

EICHLERIELLA

Eichleriella Bresadola, Ann. Myc. **1**:115. 1903.—*Hirneolina* as a section of *Sebacina* Patouillard, Essai Taxon. 24. 1900.—*Hirneolina* (Pat.) Saccardo, Syll. Fung. **17**: 208. 1905.

Fructifications coriaceous, waxy or membranaceous, subgelatinous, cup-shaped or plano-concave, rarely pendulous, hymenium typically superior, discoid, inferior in pendulous forms, even or somewhat rugulose; basidia globose-ovoid, cruciately divided, with 2–4 sterigmata; spores hyaline, cylindric, somewhat curved. It is a *Stereum* or *Cyphella* with tremellaceous hymenium.

The type species of the genus is *Eichleriella incarnata* Bres.

The original definition of *Eichleriella,* which is translated above, should be broadened to accurately describe our North American species, which are as coriaceous as *Stereum spadiceum.* All have the hymenium inferior. *Eichleriella gelatinosa* is our only species with subgelatinous hymenium.

But few species of this genus are known. Five species of *Eichleriella* have been recognized up to the present time in North America, three in Europe, and two in South America;

of our five, only one species, *Eichleriella Leveilliana,* ranges through the eastern United States; *E. spinulosa* occurs in both Europe and North America.

Key to the Species

Fructifications gray, small, ½–2 mm. long, ½–1 mm. broad, with habit of *Cyphella* ..... *1. E. Schrenkii*

Fructifications the color of raspberries and cream, and peltate at first, 1–5 cm. long, ½–1½ cm. broad ..... *2. E. Leveilliana*

Fructifications ochraceous buff, 200–300 μ thick; hymenium even; known from Cuba and Brazil ..... *3. E. alliciens*

Fructifications wood-brown, with whitish margin; hymenium dry, with tubercules like *Radulum* ..... *4. E. spinulosa*

Fructification white at first, then clay-color, tomentose, soft and spongy, ½ cm. thick; hymenium gelatinous; known from Jamaica only ..... *5. E. gelatinosa*

1. **Eichleriella Schrenkii** Burt, n. sp. Plate 27, fig. 8.

Type: in Mo. Bot. Gard. Herb. and in Farlow Herb.

Fructifications gregarious, coriaceous, sessile, pezizoid, oblong or rotund, margin free and strongly inrolled, pubescent, smoke-gray; hymenium concave, pale smoke-gray to pallid neutral gray; basidia longitudinally septate, pyriform, 22 × 11 μ; spores white in collection on slide, simple, curved, pointed at base, 12–19 × 6–7½ μ.

Fructifications ½–2 mm. long, ½–1 mm. broad, ½ mm. thick.

On bark of dead limbs of *Prosopis* (mesquite). San Antonio, Texas. February.

The general habit of this fungus resembles that of very small specimens of *Corticium Oakesii,* of large species of *Cenangium,* or of a sessile *Cyphella;* from all of which *Eichleriella Schrenkii* is easily separated by its longitudinally septate basidia which show clearly in sectional preparations. The fructifications are much smaller than those of any other species of this genus heretofore described.

Specimens examined:

Texas: San Antonio, *H. von Schrenk,* type (in Mo. Bot. Gard. Herb., 42579), and also (in Mo. Bot. Gard. Herb., 42580).

2. **E. Leveilliana** (Berk. & Curtis) Burt, n. comb. Plate 27, fig. 9.

Corticium Leveillianum Berk. & Curtis, Hooker's Jour.

Bot. 1:238. 1849.—*Stereum Leveillianum* Berk. & Curtis, Grevillea 1:163. 1873.

Type: type and cotype in Kew Herb. and in Curtis Herb., respectively.

Fructification coriaceous, soft, dry, rather thick, vinaceous fawn at first, whitening with age, resupinate-effused, with the margin free, sometimes narrowly reflexed, concolorous, minutely tomentose; hymenium composed of a surface layer about 30 μ thick of paraphyses $1\frac{1}{2}$–2 μ in diameter and of a layer of basidia under this; basidia longitudinally septate, 10–18 $\times$ 6–12 μ; spores in spore collection, white, simple, curved, pointed at base, 12–16 $\times$ 5–6 μ.

Fructifications often 5 mm. in diameter at first, finally up to 1–5 cm. long, $\frac{1}{2}$–$1\frac{1}{2}$ cm. broad, about $\frac{1}{2}$ mm. thick.

On dead limbs of several species. New York to Texas, Cuba, Jamaica, Central America, and Brazil. November to May.

This is a well-marked species upon which Berkeley made the following excellent graphic comment:

"At first forming little peltate orbicular spots, which, as they dilate, become closely attached to the matrix, with the exception of the margin, which is often free, soon confluent, soft, rather thick; of the colour of raspberries and cream. Hymenium often minutely pitted. Old specimens lose in great measure their ruddy hue, and are of a dead white."

I have seen no specimens having the hymenium minutely pitted.

Specimens examined.

Exsiccati: Ravenel, Fungi Car. II, 35.

New York: Hudson Falls, *S. H. Burnham* (in Mo. Bot. Gard. Herb., 44009, 44170, 44194); Buffalo, *G. W. Clinton.*

South Carolina: *M. A. Curtis, 1220, 92* (types and cotypes in Kew Herb. and Curtis Herb., respectively); *Ravenel,* Ravenel, Fungi Car. II, 35.

Georgia: Tallulah Falls, *A. B. Seymour,* Farlow Herb., C (in Mo. Bot. Gard. Herb., 44608).

Texas: Austin, *W. H. Long, 570,* Cornell Univ. Herb.; San

Antonio, *A. B. Langlois, bd;* same locality, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 42576).

Cuba: San Diego de los Baños, *Earle & Murrill, 296, 356 in part,* N. Y. Bot. Gard. Herb.

Jamaica: Cinchona, *W. A. & E. L. Murrill,* N. Y. Bot. Gard., Fungi of Jamaica, 493.

Brazil: Blumenau, *A. Möller,* comm. by G. Bresadola; Matto Grosso Cuyaba, *G. Malme, 599,* comm. by L. Romell.

3. **E. alliciens** (Berk. & Cooke) Burt, n. comb.

Plate 27, fig. 10.

Stereum alliciens Berk. & Cooke, Linn. Soc. Bot. Jour. **15**: 389. 1876; Massee, Linn. Soc. Bot. Jour. **17**: 201. 1891.

Type: in Kew Herb.

Fructification coriaceous, resupinate, sometimes narrowly reflexed, separable, ochraceous buff, the margin slightly paler, the reflexed portion tomentose; structure in section, 200–300 μ thick, (1) with hyphae next to substratum ochraceous, loosely interwoven and protruded, 3 μ in diameter, similar to those on outer surface of reflexed portion, (2) with intermediate layer 100–180 μ thick, composed of longitudinally arranged hyphae 2 μ in diameter, (3) with hymenium composed of basidia 10 μ below the surface, imbedded in jelly through which rise a few filiform paraphyses or hyphae to the surface; hymenium even, ochraceous buff; basidia longitudinally cruciately septate, pyriform, 12–15 $\times$ 9–10 μ; spores colorless, simple, even, curved, 10–13 $\times$ 3½–5 μ.

Fructifications of type described as several inches long, originally orbicular; Cuban specimen 1 cm. long, 1 cm. broad, reflexed side 1–2 mm. long, 1 cm. broad.

On dead wood in virgin forest. Cuba and Brazil. March.

The fructification resembles in habit and coloration that of a resupinate specimen of *Stereum hirsutum* with a very narrowly reflexed margin. The Cuban collection, of which but a single fructification was communicated to me, is much smaller than the Brazilian type and has the hyphae of the intermediate layer with gelatinously modified wall.

Specimens examined:

Brazil: San Antonio da boa vista, Rio Javary, *Traill, 1,* type (in Kew Herb.).

Cuba: San Diego de los Baños, Pinar del Rio Province, *Earle & Murrill, 405,* N. Y. Bot. Gard. Herb.

4. **E. spinulosa** (Berk. & Curtis) Burt, n. comb.

Plate 27, fig. 11.

Radulum spinulosum Berk. & Curtis, Grevillea **1**: 146. 1873. —*Radulum deglubens* Berk. & Broome, Ann. and Mag. Nat. Hist. IV. **15**: 32. 1875.—*Eichleriella deglubens* (Berk. & Br.) Lloyd, Letter No. 45: 7. 1893; Wakefield, Brit. Myc. Soc. Trans. **4**: 305. 1914.—*Stereum rufum* of English authors but not *S. rufum* Fries.—*Radulum Kmetii* Bresadola, I. R. Accad. degli Agiati Rovereto Atti III. **3**: 102. 1897.—*Eichleriella Kmetii* Bresadola, Soc. Myc. France **25**: 30. 1910.

Type: in Kew Herb.

Fructifications longitudinally and broadly effused, wood-brown, coriaceous-soft, separable, with the margin whitish, finally narrowly reflexed on the upper side and tomentose, or with margin everywhere free and curved outward; hymenium wood-brown, dry, usually bearing tubercules singly or in small clusters, with pale tips; basidia longitudinally septate, clavate, 25–36×9 μ, arranged between paraphyses with brown tips; spores simple, colorless, cylindric, curved, 15–16 × 6 μ.

Fructifications range up to 6 cm. long by 1–2 cm. wide and may be larger by confluence, about 700 μ thick; tubercules about $\frac{1}{2}$–1 mm. long.

Alabama. On bark of dead *Populus trichocarpa,* Idaho, and Oregon. July to September.

This species is distinguished by having a hymenium with configuration of a *Radulum* and cruciate basidia. The tubercules are often simple and cylindric, sometimes deformed and multifid. The wide distribution and yet the extremely local occurrence of this species together with the absence, until recently, of observations on its basidia have resulted in a very interesting synonymy. It is remarkable that this species, which occurs on *Fraxinus, Populus,* etc., in several countries of Europe, should have been collected in the United

States in Alabama, Idaho, and Oregon only. I am greatly indebted to Mr. L. Romell for a preparation from the type of *Radulum spinulosum* which makes possible the reference to this species.

Specimens examined:

Sweden: Stockholm, *L. Romell, 327,* and three unnumbered collections.

Alabama: *Peters,* Curtis Herb., 4543, preparation from type (in Kew Herb.).

Idaho: Kaniksu National Forest, Priest River, *J. R. Weir, 55.*

Oregon: Eugene, *C. J. Humphrey, 1103.*

5. E. gelatinosa Murrill, n. sp. Plate 27, fig. 12.

Type: in N. Y. Bot. Gard. Herb. and in Burt Herb.

Fructification coriaceous, effuso-reflexed, white when young, finally clay-colored, tomentose, soft to the touch, margin obtuse; context soft, spongy, zonate; hymenium tough, gelatinous, drying Hay's brown, even; basidia longitudinally septate, 13 × 11 μ; spores simple, colorless, even, flattened on one side, 8–10 × 6 μ.

Reflexed portion of fructification 1½–2 cm. long, 2½ cm. wide, ½ cm. thick.

On rotting wood in wet, wooded regions. Jamaica. December and January.

Only two collections of one fructification each were made. That of December 17 is a white, young specimen, with no basidia developed, which shows the general habit and early characters of the species, but would not have been determinable except for the later collection of January 12–14, which shows the darker coloration assumed at maturity. The thick, spongy, soft pileus of the mature fructification distinguishes this species from others known at present.

Specimens examined:

Jamaica: Troy and Tyre, Cockpit country, *W. A. Murrill & W. Harris,* N. Y. Bot. Gard., Fungi of Jamaica, 1087, type (in N. Y. Bot. Gard. Herb.), a portion in Burt Herb.; Blue

Hole, Priestman's River region, *W A. Murrill,* N. Y. Bot. Gard., Fungi of Jamaica, 180, immature specimen.

SEBACINA

Sebacina Tulasne, L. R. and C., Ann. Sci. Nat. V. **15**: 223–226. *pl. 10. f. 6–10.* 1872; Linn. Soc. Bot. Jour. **13**: 35. 1873; Brefeld, Untersuch. Myk. **7**: 102–106. *pl. 6. f. 22–26.* 1888; Patouillard, Essai Taxon. Hym. 24, 25. 1900 (with the exclusion of section *Hirneolina*).—*Exidiopsis* Brefeld, Untersuch. Myk. **7**: 94. *pl. 5. f. 20–22.* 1888.—*Stypella* Möller, A., Bot. Mitth. a. d. Tropfen. 8, Protobasidiomyceten 166. *pl. 4. f. 6, 7.* 1895.

Fructification coriaceous, membranaceous or floccose, gelatinous, waxy or pulverulent, resupinate, with habit of *Corticium;* basidia longitudinally septate, close together or scattered, sometimes between bushy conidiophores; spores colorless, producing in germination a similar spore or a cluster of conidia.

The type species of the genus is *Corticium incrustans* Pers.

Sebacina incrustans occurs sometimes on the ground and incrusting herbaceous stems and various erect objects but is often on decaying wood; *S. Helvelloides* occurs on the ground and incrusting erect objects; *S. chlorascens* has been observed incrusting the mossy bases of living trees; the other species have been recorded only on dead wood and bark. A few members of this genus are thick and spongy and were originally included in *Thelephora;* usually the species are thin and *Corticium*-like in general habit and were in several instances published under *Corticium.* In the dried conditions some species of *Sebacina* may be tentatively recognized as such by having the hymenial surface glassy or resembling dried cartilage; but such a separation from *Corticium* is very uncertain, for some species of *Sebacina* dry with a dull, soft surface and some true Corticiums assume the appearance of dried cartilage in drying.

It seems probable that it will always be difficult to determine resupinate species of *Hymenomycetes;* it is not possible to do so from the descriptions alone of the earlier botanists. European authors have recently been enlarging such descrip-

tions by giving spore characters, dimensions of basidia, cystidia, and hyphae, and the presence or absence of clamp connections. Such additional characters may often be obtained quickly by microscopic examination of a portion of the fructification which has been teased out and crushed down in dilute potassium hydrate solution; by these helpful additional characters, some species may be recognized with reasonable accuracy, but there are comparatively few such species. Structure in section of the fructification affords important characters for the identification of resupinate species. In practical work with these species, a microscopical mount of a sectional preparation of a type specimen is the next best thing for purposes of comparisor to having the type itself.

My method of determining a resupinate specimen is to observe closely its general habit and characters, such as consistency, adnation, thickness, surface, margin, substratum, and color. Color is an important character when given in terms of an adequate color standard. The color which the specimens retain in drying is often the only color character available; it is more constant, fortunately, than is commonly appreciated, for it has to be the color factor in the comparison of herbarium specimens. The preliminary observation may suggest that the species is one of several of somewhat similar habit which may be of the same genus or of various genera. The sectional preparations, which are now made, may present (*a*) a uniform, homogeneous arrangement of similar hyphae from substratum to hymenium, (*b*) dissimilar hyphae or organs distributed uniformly throughout the whole fructification, (*c*) a layered, heterogeneous arrangement of various types with the layers more or less sharply differentiated from one another, (*d*) a stratose arrangement having the first stratum extend from the substratum to the upper surface of the first hymenium, the second stratum a repetition of the first and borne on the first, and so on. Under *a* there are characteristic varieties of structure, constant for each species, such as all the hyphae in erect position extending from substratum to hymenial surface, or all interwoven, or all procumbent, and there are also constant

differences in regard to whether the hyphae are crowded close together or are loosely arranged. Under *c*, a conspicuous example would be one in which the layer next to the substratum is composed of longitudinally arranged hyphae (that is, parallel with the substratum) crowded closely together; from this layer, a few branches might extend outward at right angles to the first layer and form a layer of loosely arranged, erect hyphae — the second layer; the hyphae of the second layer might branch abruptly at its outer surface and bear a compact hymenial layer. Some species invariably form a loosely interwoven layer next to the substratum, and on the surface of this layer form a dense hymenial layer, as, for example, *Sebacina incrustans, S. chlorascens,* and *S. Helvelloides.* Sterile fructifications may frequently be determined by their general characters and structure in section.

The preparations which reveal structure in section, give also spores, basidia, paraphyses, and other organs. From the combination of general characters, structure in section, and details of spores and noteworthy organs, the species becomes manifest. Our species of *Sebacina* are described in accordance with this method in the following pages.

Key to the Species

Fructifications on the earth, running up and incrusting the bases of living stems and trunks as well as dead objects 1
Fructifications confined to bark and wood of dead branches and trunks ... 2

1. Sometimes passing into branches or ascending flaps; hymenial layer drying warm buff, 60–150 μ thick; paraphyses densely crowded and somewhat interwoven or adglutinated ..... *1. S. incrustans*
1. Pileate branches drying cream-color with a glaucous tint, imbricated, the apices spiculose or fimbriate; hymenial layer drying vinaceous brown, 140–240 μ thick ..... *3. S. chlorascens*
1. Not forming free branches or flaps; hymenial layer 200–300 μ thick; paraphyses straight and rod-like; basidia 20–25×15 μ ..... *4. S. Helvelloides*
2. Fructifications white or whitish when dry 3
2. Fructifications not white 4
3. Hymenium composed of unbranched, flexuous, even-walled, deeply staining, clavate organs 40–45×6 μ, in addition to few-branched paraphyses and basidia ..... *5. S. Shearii*
3. Hymenium composed of paraphyses and basidia; fructification 300–400 μ thick; margin thick, not closely adnate to substratum ..... *6. S. macrospora*
3. Hymenium composed of basidia and paraphyses; fructification 50–150 μ thick, shining white at first; margin very thin and closely adnate .. *7. S. calcea*
3. Hymenium composed of basidia and paraphyses; fructification 200–300 μ thick, dirty whitish; hyphae incrusted in upper two-thirds of fructification; margin thin and closely adnate ..... *8. S. monticola*

4. Drying ochroleucous, basidia at or near the surface in tissue not sharply differentiated as a layer from tissue near substratum; much crystalline matter about 100 μ below surface. On *Alnus*, South Carolina ..... *9. S. scariosa*
4. Drying some variety of brown 5
4. Drying fuscous to black 6

5. Drying cacao-brown (testaceous of Saccardo's 'Chromotaxia'); separable from substratum; resembling *S. incrustans* but with margin soon detached and spores 6–7×4½–5 μ. On juniper, Alabama ..... *2. S. deglubens*

5. Blue-purple when fresh, drying tawny olive to Saccardo's umber where directly on the wood; adnate to substratum; 30–45 μ thick; basidia 7–10×6–8 μ; spores 6–7×3–5 μ ..... *10. S. podlachica*

5. Drying cinnamon-brown; adnate to substratum; 100–140 μ thick; scattered paraphyses with bushy-branched, brown tops rise 45–60 μ above the basidia. On *Magnolia*, Delaware ..... *11. S. cinnamomea*
6. Hay's brown when moist, drying fuscous, the margin pale cartridge-buff; separable from substratum; 500–600 μ thick. On *Populus*, Idaho ..... *12. S. adusta*
6. Drying blackish plumbeous; adnate to substratum; 150–200 μ thick, the margin indeterminate. On *Populus*, Washington ..... *13. S. plumbea*
6. Grayish when moist, drying dark mouse-gray and shining; adnate to substratum; 50–160 μ thick, the margin indeterminate. On very rotten wood, New England ..... *14. S. atrata*

1. Sebacina incrustans Pers. ex Tulasne, Ann. Sci. Nat. Bot. V. **15**: 225. *pl. 10. f. 6–10.* 1872; Linn. Soc. Bot. Jour. **13**: 36. 1873. Plate 27, fig. 13.

Corticium incrustans Persoon, Obs. Myc. **1**: 39. 1796.—*Thelephora incrustans* Persoon, Syn. Fung. 573. 1801; Fries, Syst. Myc. **1**: 448. 1821.—*Thelephora sebacea* Persoon, Myc. Eur. **1**: 155. 1822; Fries, Elench. Fung. **1**: 214. 1828; Hym. Eur. 637. 1874; Saccardo, Syll. Fung. **6**: 540. 1888.—*Corticium sebaceum* Massee, Linn. Soc. Bot. Jour. **27**: 127. 1891.—*Merisma cristatum* Persoon, Syn. Fung. 583. 1801.—*Thelephora cristata* Fries, Syst. Myc. **1**: 434. 1821; Hym. Eur. 637. 1874; Saccardo, Syll. Fung. **6**: 539. 1888.—*Sebacina incrustans* Tul. ex Bresadola, in part (Hym. Hung. Kmet.), I. R. Acad. Sci. Agiati III. **3**: 117. 1897.

Illustrations: Tulasne, *loc. cit.*—Persoon, Com. Fung. Clav. *pl. 4. f. 4;* Berkeley, Outlines Brit. Fung. *pl. 17. f. 6;* Brefeld, Untersuch. Myk. **7**: *pl. 6. f. 22–26.* Hennings in Engl. & Prantl, Nat. Pflanzenfam. (**I.** 1 **)**: 91. *f. 59 C, D;* Nees, System *pl. 34. f. 256 B;* Patouillard, Tab. Anal. Fung. *f. 155;* and Essai Tax. Hym. 25. *f. 17 a, b;* Soc. Myc. Fr. Bul. **5**: *pl. 7. f. 11.*—See Saccardo, Syll. Fung. **20**: 945 for references to some additional illustrations which I have not seen.

Type: authentic specimens of *Thelephora incrustans* and *Merisma cristatum* from Persoon in Kew Herb.

Fructifications coriaceous-fleshy, varied in form, creeping on the ground and ascending and incrusting small erect objects and forming little columns and free branches, the apices somewhat awl-shaped or fringed, or effused and resupinate on bark, whitish, drying warm buff; structure in section, 250–400 μ thick, (1) with a broad layer of very loosely interwoven rather stiff hyphae, 2–2½ μ in diameter, which divide above into fine branches and form (2) a densely interwoven layer about 60–150 μ thick with the basidia in the upper 40–90 μ among the very fine (1½ μ in diameter), densely crowded, somewhat interwoven filaments from the subhymenium; basidia longitudinally septate, ovoid or pyriform, 12–20 × 9–14 μ; spores colorless, simple, even, flattened on one side or curved, 12–14 × 6–8 μ.

Fructifications 5–6 cm. long, 2–5 cm. wide, ascending objects 2–5 cm.; pileate flaps, when present, ½–1 cm. long.

On the ground in woods and incrusting objects, and resupinate on logs. Canada to Louisiana and westward to Missouri. June to October. Common.

S. incrustans is the common incrusting *Sebacina* of Eastern North America. It may usually be recognized at sight by coriaceous-fleshy consistency, occurrence on earth and running up and incrusting living objects, and pallid color. The thinner hymenial layer, paraphyses less rod-like in form, and finer and thinner-walled hyphae of layer next to the substratum afford structural characters separating specimens of this species from those of *S. chlorascens* and *S. Helvelloides.*

I exclude from the synonymy of *S. cristata, Clavaria laciniata* of Bulliard's 'Hist. Champ.' 1: 208. *pl. 415. f. 1,* because in the absence of authentic specimens and observations in regard to spores and basidia, it is not certain that *C. laciniata* Bull. is *Merisma cristatum.* Bulliard's figures represent quite as well an incrusting European fungus communicated to me by Bresadola under the name *Thelephora fastidiosa* (Pers.) Fr., which has simple basidia and colorless echinulate spores. This species is the *Thelephora cristata*

of Patouillard's 'Tab. Anal. Fung.' No. 559, and *Cristella cristata* of his 'Essai Taxon. Hym.' *f. 28.* Patouillard notes that *Clavaria laciniata* is a synonym of the species which he figures. Because of the uncertainty as to whether figures of *Thelephora cristata* by European authors represent the true *Merisma* [*Sebacina*] *cristatum* Pers. or the echinulate-spored *T. fastidiosa* (Pers.), I have refrained from citing any illustrations except that of Persoon, of whose species I have studied an authentic specimen.

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 513.

The specimen in Thuemen, Myc. Univ. 2009, under the name *Thelephora sebacea,* collected in France, is *Thelephora mollissima* Pers.

Europe: authentic specimens of *Thelephora incrustans* and *Merisma cristatum* from Persoon in Kew Herb.

Sweden: sterile specimen determined as *Thelephora cristata* by E. Fries (in Fries Herb.); Stockholm, *L. Romell, 54.*

Canada: *J. Macoun, 5, 10.*

Quebec: Hull, *J. Macoun, 203, 313.*

Ontario: near Ottawa, *J. Macoun, 40* (in Can. Geol. Surv. Herb.); London, *J. Dearness.*

Maine: Portage, *L. W. Riddle.*

New Hampshire: Shelburne, *W. G. Farlow* (in Farlow Herb.).

Vermont: Middlebury, *E. A. Burt,* two collections.

Massachusetts: Williamstown, *W. G. Farlow* (in Farlow Herb.).

New York: Hudson Falls, *S. H. Burnham, 2* (in Mo. Bot. Gard. Herb., 43995).

Pennsylvania: *Michener, 5821* (in Curtis Herb.); Trexlertown, *W. Herbst.*

District of Columbia: Rock Creek, *C. L. Shear, 793.*

North Carolina: Asheville, *H. C. Beardslee, 03126.*

South Carolina: *Ravenel, 1619* (in Curtis Herb.).

Louisiana: St. Martinville, *A. B. Langlois, F, 2015;* the same locality and collector, (3022 in Lloyd Herb.); Baton Rouge, *Edgerton & Humphrey, 667.*

Ohio: *A. P. Morgan* (in Lloyd Herb., 2655, 2656); Cincinnati,

C. G. Lloyd, 4198; Loveland, *D. L. James* (in U. S. Dept. Agr. Herb.).

Wisconsin: Blue Mounds, *E. T. and S. A. Harper, 864, 879, 880;* Madison, *W. Trelease* (in Mo. Bot. Gard. Herb., 5145, 44779); *C. J. Humphrey, 2146* (in Mo. Bot. Gard. Herb., 44784).

Illinois: Riverside, *E. T. and S. A. Harper, 698.*

Missouri: Creve Coeur, *E. A. Burt* (in Mo. Bot. Gard. Herb., 44763).

2. **S. deglubens** (Berk. & Curtis) Burt, n. comb.

Corticium deglubens Berk. & Curtis, Grevillea **1**: 166. 1873.

Type: type and cotype in Kew Herb. and Curtis Herb.

Fructification resupinate, effused, coriaceous, separable, white beneath, drying about cacao-brown, the margin very narrow, white, byssoid, soon detached; structure in section 250–300 μ thick, (1) with a very loosely interwoven layer 180–200 μ thick, having hyphae $1\frac{1}{2}$–2 μ in diameter which branch and form (2) a very densely interwoven layer 80 μ thick with the basidia in the upper 30 μ, not quite reaching to the surface, among the very fine, densely interwoven filaments from the subhymenium; basidia longitudinally septate, 15 $\times$ 10–12 μ; spores colorless, simple, even, flattened on one side, 6–7 $\times$ $4\frac{1}{2}$–5 μ.

On juniper, Alabama.

This fungus has the same type of structure which is found in resupinate specimens of *Sebacina incrustans.* It differs from the latter in having the hymenium darker, all the spores found in a sectional preparation a little smaller, and the hyphae of the layer next to the substratum a little smaller and more flaccid than those of *S. incrustans,* and the margin was described as soon detached. These differences may be merely the variation from specific type of a single collection, or they may be those of a subspecies of *S. incrustans* which has taken on the saprophytic life on dead wood, prevalent for most species of *Sebacina.* Until other collections, referable to *S. deglubens* are made, the former view appears the more probable.

Specimens examined:
Alabama: *Peters,* Curtis Herb., 4557, type (in Kew Herb.).

3. **S. chlorascens** Burt, n. sp. Plate 27, fig. 15.

Type: in Mo. Bot. Gard. Herb. and in Farlow Herb.

Fructification coriaceous, drying cream-color with glaucous tint, effused, ascending and incrusting the mossy bases of trees and forming imbricated, free, pileate, sterile branches, the apices spiculose or fimbriate; hymenium gelatinous, drying vinaceous brown, occurring in somewhat scattered spots on the lower portions of the fructification; structure in section 800 μ thick, with (1) a broad, spongy layer next to the substratum of loosely interwoven, rather rigid, even-walled hyphae $2\frac{1}{2}$–3 μ in diameter, which bear (2) a sharply differentiated hymenial layer 140–240 μ thick, composed of rod-like paraphyses 2 μ in diameter, between which occur basidia throughout the outer 60 μ of the layer; basidia longitudinally septate, pyriform, 15–18×12 μ; spores simple, colorless, flattened on one side, 10–$10\frac{1}{2}$× 6–7 μ.

Fig. 1
S. chlorascens
Paraphyses,
basidium×540.

Ascending objects 2–4 cm., 1–2 cm. broad; free branches up to 5 mm. long.

On mossy bases of living trees. Florida. Autumn.

As shown by the figures in pl. 27, the pileate branches of *S. chlorascens* do not resemble those of *S. incrustans.* The structure in section is different in every detail from that of specimens of the latter species and approaches more closely that of *S. Helvelloides,* but the fructification is thinner than that of the latter, has smaller basidia and spores, and the basidia distributed from the surface to about 60 μ below the surface, and forms free pileate branches.

Specimens examined:
Florida: Cocoanut Grove, *R. Thaxter, 98,* type (in Mo. Bot. Gard. Herb., 43923, and in Farlow Herb.).

4. **S. Helvelloides** (Schw.) Burt, n. comb. Plate 27, fig. 14.

Thelephora Helvelloides Schweinitz, Naturforsch. Ges. Leipzig Schrift. **1**: 108. 1822; Am. Phil. Soc. Trans. N. S. **4**: 168. 1834; Fries, Elenchus Fung. **1**: 193. 1828; Epicr. 541. 1836–1838.—*Corticium Helvelloides* Massee, Linn. Soc. Bot. Jour. **27**: 153. 1891.—*Corticium basale* Peck, N. Y. State Mus. Rept. **43**: 69 (23). 1890.

Type: in Herb. Schw. and portions in Curtis Herb. and in Kew Herb.

Fructification coriaceous, spongy, effused, convex, closely adnate and incrusting, on ground in mosses and on bark at bases of living trees, at first whitish, drying honey-color to warm buff; structure in section, with (1) a very thick spongy layer next the substratum, of loosely interwoven, branched, rather rigid, even-walled, brownish hyphae, 3–3½ μ in diameter, which bear (2) a fertile layer 200–300 μ thick made up of great numbers of erect, straight, cylindric paraphyses 2 μ in diameter, between which occur the basidia at about 40–50 μ below the surface; basidia longitudinally septate, pyriform, 20–25 $\times$ 15 μ; spores colorless, simple, flattened or slightly curved on one side, 12–13 $\times$ 6 μ.

Fructifications 3–15 cm. long and wide, drying about ½–2 mm. thick to 9 mm. thick in type which covers a cushion of moss plants.

On ground and bark at bases of living trees. New York to North Carolina. July and August.

Specimens of this species have somewhat the habit of thick specimens of *Coniophora puteana* but are of very different structure. The abundant, erect, unbranched, cylindric paraphyses often 200 μ long which compose the greater part of the hymenium, and the large basidia are reliable characters for identifying *Sebacina Helvelloides* when sections are studied; the coarser and colored hyphae of the species give an additional character separating it from *S. incrustans* when the latter occurs strictly resupinate.

The type specimen is abnormal in thickness and ridged surface by running over and incrusting a bed of moss. The hanging rootlets referred to in the original description are

moss stems. The specific name is rather fanciful and misleading.

Specimens examined:

New York: Whitehall, *C. H. Peck,* type of *Corticium basale* (in Coll. N. Y. State); Alcove, *C. L. Shear, 1221.*

North Carolina: Salem, *Schweinitz,* type (in Herb. Schw., in Curtis Herb., and in Kew Herb.).

5. S. Shearii Burt, n. sp. Plate 27, fig. 16.

Type: in Burt. Herb., and in Shear Herb.

Fructification coriaceous, effused, dull white, drying pale olive-buff, cracked, the margin determinate, entire; structure in section, 140–200 μ thick, with (1) a broad and dense layer next to the substratum of longitudinally arranged, slightly brownish, even-walled hyphae 1½–2 μ in diameter, which branch and curve outward at a right angle and form (2) a fertile, less compact layer 60–75 μ thick of suberect, few-branched paraphyses 3 μ in diameter, of basidia at about 15–20 μ below the surface, and of scattered, even-walled, flexuous, cylindric-clavate organs—perhaps gloeocystidia—40–45 × 6 μ, not emergent above the surface; basidia longitudinally septate, pyriform, 15 × 9 μ, with sterigmata 18 × 3 μ; spores colorless, simple, curved, 9–15 × 4½–6 μ.

Fig. 2 S. Shearii. Paraphysis at left, organ×540.

Fructifications in crevices of bark at first, 2 × 1 mm., at length, by confluence, 7 cm. long, 1 cm. broad.

On dead *Berberis vulgaris.* District of Columbia. October.

This species is well characterized by the presence in the hymenial layer of flexuous, even-walled organs, which are either latex or gloeocystidia, and by the broad layer of longitudinally arranged hyphae which shows relationship to *Eichleriella,* although the margin is not distinctly free. A few small granules are present on the hymenial surface but I do not know that they are a constant character.

Specimens examined:

District of Columbia: grounds U. S. Dept. Agr., Washington, *C. L. Shear, 1238,* type.

6. S. macrospora (E. & E.) Burt, n. comb.

Corticium macrosporum Ell. & Ev., Torr. Bot. Club Bul. 27:49. 1900.

Type: in N. Y. Bot. Gard. Herb.; specimens from type collection in Lloyd Herb., and in Burt Herb.

Fructification coriaceous, appressed, thin, dull white, cracked, the narrow, white, cottony margin sometimes narrowly involute; structure in section, 300–400 μ thick, with (1) a very broad layer of longitudinally arranged and somewhat obliquely ascending crowded hyphae $1\frac{1}{2}$ μ in diameter, colorless next to substratum but brownish in upper part of layer, which pass into (2) the hymenial layer 60–100 μ thick, consisting of erect, bushy paraphyses and of basidia; basidia longitudinally septate, pyriform to subglobose, 15×9–12 μ; spores colorless, simple, flattened on one side or curved, $10\frac{1}{2} \times 4\frac{1}{2}$–$6$ μ.

Appearing at first in orbicular patches 3–5 mm. in diameter, at length confluent and up to 4 cm. long, $1\frac{1}{2}$ cm. broad.

On pine (*Pinus Strobus*) limbs. Ohio. September.

This species is near *Sebacina calcea*, but the single collection which has been studied seems distinct from the latter by the thick, determinate margin, sometimes free and slightly upturned, by the greater thickness of the fructifications, by the brown hyphae of the middle region, and by walls of hyphae not gelatinously modified as in *S. calcea*. A relationship to *Eichleriella* is manifest in the broad layer of longitudinally arranged hyphae and in the tendency of the margin to be slightly free. The original description gives this species as on *"Fraxinus?"*, but the limbs are *Pinus strobus*. The spores are not exceptionally large; the specific name was probably based on immature basidia.

Specimens examined.

Ohio: Linwood, *C. G. Lloyd, 3113*, type collection.

7. S. calcea (Pers.) Bresadola, Fungi Tridentini 2:64. *pl. 175*. 1892. Plate 27, fig. 17.

Thelephora calcea Persoon, Syn. Fung. 581. 1801; Myc. Eur. 1:153. 1822.—*Thelephora calcea c. albido-fuscescens*

Fries, Elenchus Fung. 1: 215. 1828.—*Thelephora acerina* forma *Abietis* Fries, Syst. Myc. 1: 453. 1821.—*Corticium Abietis* (Fr.) Romell, Bot. Not. 1895: 72. 1895.—*Xerocarpus farinellus* Karsten, Finska Vet.-Soc. Bidrag 37: 139. 1882.

Illustrations: Bresadola, *loc. cit.;* Patouillard, Essai Taxon. Hym. 25. *f. 17b.*

Fructification effused, closely adnate, crustaceous, slightly pulverulent, shining white at first, at length darkening in the central portion from cartridge-buff to pale drab-gray, cracked, the margin much thinner and farinaceous; structure in section, 50–150 μ thick, (1) with hyphae next the substratum interwoven, 2 μ thick, the wall gelatinously modified, (2) hymenial layer 40–60 μ thick, composed of basidia and of paraphyses branched at the apex into very fine branches loaded with minute granules; basidia more abundant in the lower portion of the hymenial layer, longitudinally septate, 14 × 9 μ; spores colorless, simple, cylindric, curved, 8–12 × 4–5 μ.

Fig. 3
S. calcea.
Paraphyses × 540.

Fructifications 3–9 cm. long, 1–3 cm. broad.

On bark and wood of dead branches of spruce, pine, hemlock, white cedar, oak, ash, elm, maple, and elder. Canada, northern New England, and New York to Georgia, and in Washington. March to January—perhaps throughout the year.

As good distinctive macroscopic characters this species has: chalky white color with central portions ashy; powdery surface under a lens; thinness on drying and margin still thinner, so that it appears mealy under a lens rather than membranous. The fine branches and granules at the tips of the paraphyses show best in lactic acid preparations; potassium hydrate solution has a solvent action here. I have not been able to study an authentic specimen of *Thelephora calcea* Pers. and accept Bresadola's conclusion on this point.

Specimens examined:

Exsiccati: Romell, Fungi Exs. Scand. 129.

Austria: *G. Bresadola.*

Sweden: *L. Romell, 58, 59;* Stockholm, *L. Romell,* Fungi Exs.

Scand. These specimens are under the name *Corticium Abietis.*

Norway: Christiania, *M. N. Blytt* (in Herb. Fries and determined by Fries as *Corticium calceum*).

Finland: Mustiala, *P. A. Karsten,* under the name *Xerocarpus farinellus.*

Canada: *J. Macoun, 30, 33.*

New Hampshire: Chocorua, *W. G. Farlow,* two collections.

Vermont: Middlebury, *E. A. Burt,* two collections; Ripton, *E. A. Burt;* Little Notch, Bristol, *E. A. Burt.*

New York: Alcove, *C. L. Shear, 1134, 1208;* Hague, *C. H. Peck, 10;* Clear Water, *G. F. Atkinson,* Cornell Univ. Herb., 5049.

Georgia: Tipton, *C. J. Humphrey, 177;* Savannah, *C. J. Humphrey, 5106* (in Mo. Bot. Gard. Herb., 15081).

Washington: Bingen, *W. N. Suksdorf, 695, 711, 763, 765, 864.*

8. **S. monticola** Burt, n. sp.

Type: in Mo. Bot. Gard. Herb.

Fructification coriaceous, resupinate, cracked, dirty whitish approaching pale smoke-gray, the margin closely adnate; structure in section 200–300 μ thick, with hyphae colorless, 3–4 μ in diameter, ascending obliquely from substratum to surface, densely crowded together, more interwoven and little incrusted in the lower third of the fructification, but more loosely arranged and heavily incrusted in the whole upper two-thirds, terminating in incrusted paraphyses which are either simple or 2–4-branched and with the hyphal body about $2\frac{1}{2}$ μ in diameter under the incrustation; basidia about 40 μ below the surface of the hymenium, longitudinally septate, 15–20 $\times$ 9–12 μ; spores simple, colorless, even, cylindric, straight or curved, 9–$10\frac{1}{2}$ $\times$ 5–$5\frac{1}{2}$ μ.

The portion of the fructification described is 5 cm. long, about $1\frac{1}{2}$ cm. wide.

On bark of log of *Picea Engelmanni,* altitude 8,500 ft., Pike's Peak, Colorado. August.

This species belongs in the group with *Sebacina calcea* and *S. macrospora;* it is distinguished from both of these by the

incrustation of its hyphae and by simpler paraphyses, which are either unbranched or with only about 2–4 branches not branching repeatedly and becoming so attenuated as to be nearly invisible except for the granules which they bear.

Specimens examined:

Colorado: Pike's Peak, *G. G. Hedgcock,* comm. by C. J. Humphrey, 2571, type (in Mo. Bot. Gard. Herb., 15157).

9. S. scariosa (Berk. & Curtis) Burt, n. comb.

Corticium scariosum Berk. & Curtis, Grevillea **2**: 3. July, 1873.—*Corticium secedens* Saccardo, Syll. Fung. **6**: 635. 1888.

Type: type and cotype in Kew Herb. and Curtis Herb., respectively.

"Forming a thin, oblong, membranous stratum, without any distinct border; hymenium pulverulent ochroleucous."

—Original description.

Structure in section 300–600 μ thick, with hyphae 2 μ in diameter, branched, very loosely interwoven, extending from substratum to basidia, with walls gelatinously modified, imbedded in jelly, much crystalline matter about 90–120 μ below the hymenial surface; basidia at or near the surface, longitudinally septate, pyriform to subglobose, 12–15 × 9–12 μ; no spores found.

On alder, South Carolina.

The type specimens of this species have the general habit of *Peniophora gigantea,* which they also resemble in being separable and in cracking and peeling up from the substratum, but they are more lemon-yellow in color than specimens of the latter species. The structure in section is distinctive and suggestive of that of *Eichleriella alliciens.* Authors have sometimes confused *Corticium scariosum* B. & C. with *Corticium scariosum* B. and Br., published from Ceylon a few months later in the same year. The types of these fungi are not of the same genus, the American specimens having longitudinally septate basidia.

Specimens examined:

South Carolina: Society Hill, *M. A. Curtis, 4916* (type and cotype in Kew Herb. and Curtis Herb.).

10. S. podlachica Bresadola, Ann. Myc. 1:117. 1903.

Type: in Bresadola Herb. and a portion in Burt Herb.

Fructification effused, closely adnate, described as "e pallido-caerulea caesio-hyalina," drying tawny olive to Saccardo's umber where directly on the wood; structure in section 30–45 μ thick, with hyphae 2 μ in diameter closely crowded together and rising obliquely from substratum to the surface; basidia in upper 15 μ of fructification among the hyphal filaments, longitudinally septate, pyriform, 7–10 $\times$ 6–8 μ; spores colorless, simple, even, curved, 6–7 $\times$ 3–5 μ.

Covering areas 5 cm. long, 2 cm. broad.

On decaying coniferous wood, Massachusetts; on decaying beech wood, Russian Poland.

The Massachusetts collection was noted as blue-purple when fresh; in some places algae coating the wood have been covered by the fructification and the modified color of this algal layer is seen through the dried fructification; where the fungus coats the wood directly, the color of specimens which have been several years in the herbarium is the tawny olive. The American collection agrees closely with that communicated by Bresadola.

Specimens examined:

Russian Poland: on beech wood, *Eichler,* comm. by Bresadola, part of type.

Massachusetts: on coniferous wood, *W. G. Farlow.*

11. S. cinnamomea Burt, n. sp. Plate 27, fig. 18.

Type: in Burt Herb.

Fructification effused, coriaceous, dry, closely adnate, drying cinnamon-brown, the margin determinate, thick, entire; structure in section 100–140 μ thick, with (1) a layer 10–30 μ thick next to the substratum of longitudinally arranged, densely interwoven hyphae 2–2½ μ in diameter, which bear (2) the hymenial layer composed of basidia at the lower side of the layer, and of loosely arranged, highly branched, bush-

Fig. 4
S. cinnamomea.
Paraphysis×540.

shaped paraphyses with brown branches of zigzag form, which rise 45–60 μ above the basidia and give the characteristic color of the hymenium; basidia 15–20 × 9–11 μ, longitudinal septation not positively made out; no spores found; paraphyses 75 μ long, trunk 1½–2 μ in diameter, sweep of branched top about 20 μ.

Fructification 4 cm. long, 1 cm. broad.

On limbs of dead *Magnolia glauca*. Maryland. December.

It is not certain that this fungus is a *Sebacina*, for none of its basidium-like organs show longitudinal septa, although in a very few there is arrangement of the protoplasm suggestive of such septation. The specimen is a little too immature for generic reference but is probably a young *Sebacina* in my opinion. The species is distinct from others in possible genera by cinnamon-brown color, paraphyses scattered as to trunks but with such brown, bushy-branched tops as to form a compact surface of the color stated.

Specimens examined:

Maryland: Takoma Park, *C. L. Shear, 1339*, type.

12. S. adusta Burt, n. sp. Plate 27, fig. 19.

Type: in Burt Herb.

Fructification broadly effused, coriaceous, separable from the substratum, Hay's brown when moist, drying fuscous, the margin pale cartridge-buff, fibrillose-fimbriate; structure in section, 500–600 μ thick, composed of densely interwoven and obliquely ascending hyphae 3 μ in diameter, the walls not gelatinously modified, which bear the basidia at the surface of the hymenium; basidia longitudinally septate, pyriform, 12–16 × 8–10 μ; spores colorless, simple, curved, 10–12 × 4–5 μ.

Fig. 5
S. adusta.
Basidia and
hyphae×540.

Fructification 12 cm. long, 4 cm. broad.

On decorticated trunk of *Populus trichocarpa*. Idaho. July to September.

In the single collection of this species which has been received the margin is everywhere closely applied to the substratum and shows no tendency towards becoming free or

reflexed, hence the species must be included in *Sebacina*. The distinguishing specific characters are easy separation as an unbroken membrane of the moist fructification from the substratum, thickness of fructification, and position of the basidia at the surface of the hymenium.

Specimens examined:

Idaho: Kaniksu National Forest, Priest River, *J. R. Weir, 12,* type.

13. S. plumbea Burt, n. sp. Plate 27, fig. 20.

Type: in Burt Herb.

Fructification effused, closely adnate, drying blackish plumbeous, pruinose, the margin indeterminate; structure in section, 150–200 μ thick, with (1) a broad layer next to the substratum containing much crystalline matter in the interspaces between the interwoven suberect hyphae $1\frac{1}{2}$–2 μ in diameter, the wall gelatinously modified, and (2) a hymenial layer about 60 μ thick consisting of basidia, and of hyphae which branch and form a densely interwoven hymenial surface; basidia about 30 μ below the surface of hymenium, longitudinally septate, pyriform, 15–18 $\times$ 10–13 μ; spores colorless, simple, even, curved, 13–15 $\times$ $4\frac{1}{2}$–6 μ.

Fig. 6
S. plumbea. Basidia and hyphae×540.

Fructification 4–8 cm. long, $\frac{1}{2}$–1 cm. broad.

On blackened wood of *Populus trichocarpa*. Washington. November.

The coloration and habit of specimens of this species agree closely with those of the European *Corticium plumbeum* Fr. which have been received from Karsten, but the internal structure is wholly different from that of the latter.

Specimens examined:

Washington: Bingen, *W. N. Suksdorf, 862,* type.

14. S. atrata Burt, n. sp. Plate 27, fig. 21.

Type: in Burt Herb. and in Farlow Herb.

Fructification effused, somewhat gelatinous, closely adnate, grayish when moist, drying dark mouse-gray and shining, the margin thinning out and indeterminate; structure in section,

50–160 μ thick, with even-walled hyphae 3 μ in diameter, densely interwoven next to the substratum, then curving outward to form a hymenial layer 50–90 μ thick, consisting of erect, parallel, rod-like paraphyses 2 μ in diameter and of basidia about 30 μ below the surface of the hymenium; basidia longitudinally septate, pyriform, about 18×12 μ; spores colorless, simple, somewhat flattened on one side, $8–10 \times 6–7$ μ.

Fig. 7
S. atrata.
Paraphysis,
basidia×540.

Fructifications $2\frac{1}{2}$ cm. long, $1\frac{1}{2}$ cm. broad.

On very rotten coniferous and frondose wood. New Hampshire and Massachusetts. May.

When bits of dried specimens of this species are moistened, they become softer and more gelatinous than is usual with those of other species of the genus, but walls of the hyphae do not show gelatinous modification in sectional preparations. The paraphyses are as noteworthy as those of *Sebacina Helvelloides*, being arranged close together side by side in a palisade layer. They are sometimes simple rods, sometimes divided into equal branches which rise side by side to the surface of the hymenium.

Specimens examined:

New Hampshire: Chocorua, *W. G. Farlow*, two collections (of which No. *a* is in Mo. Bot. Gard. Herb., 44782).

Massachusetts: Magnolia, *W. G. Farlow*, type.

(To be continued.)

Explanation of Plate

PLATE 26

The figures of this plate have been reproduced natural size from photographs of dried herbarium specimens.

Fig. 1. *Tremellodendron Cladonia.* *a*, from specimen collected in Canada by J. Macoun, 78; *b*, collected at Hague, New York, by C. H. Peck, 7; *c*, collected at Cincinnati, Ohio, by A. P. Morgan, Lloyd Herb., 32.

Fig. 2. *T. Cladonia*, from the type of *Thelephora gracilis*, collected in Alabama by F. S. Earle, 13.

Fig. 3. *T. candidum.* Collected at Newfane, Vermont, by C. D. Howe. *a* agrees closely with the type and is my standard for comparison; *b* could be separated without fracture into three portions, each having form of *a*.

Fig. 4. *T. merismatoides.* *a*, from specimen collected at York County, Pennsylvania, by N. M. Glatfelter; *b*, single fructification from the cluster *a*; *c*, from a very fasciculate specimen having stems grown together and branches still fimbriate at apex, collected at Haddonfield, New Jersey, by T. J. Collins.

Fig. 5. *T. simplex.* From type collected in Porto Rico, by J. R. Johnston. The fructification on the right is inverted.

Fig. 6. *T. pallidum.* *a*, from specimen collected at Middlebury, Vermont, by E. A. Burt; *b*, from specimen in Mo. Bot. Gard. Herb., 712370, collected at St. Louis, Missouri, by N. M. Glatfelter. Both show the growth together of the flattened pileate divisions.

Fig. 7. *T. tenue.* *a*, from type, collected at Chester Vale, Jamaica, by W. A. and E. L. Murrill, 400; *b*, from specimens collected at Cinchona, Jamaica, by the same collectors, 614.

BURT—THELEPHORACEAE OF NORTH AMERICA

1 AND 2. TREMELLODENDRON CLADONIA.—3. T. CANDIDUM.—4. T. MERISMATOIDES.—5. T. SIMPLEX.—6. T. PALLIDUM.—7. T. TENUE.

Explanation of Plate

PLATE 27

The figures of this plate have been reproduced natural size from photographs of dried herbarium specimens, except in the cases noted otherwise.

Fig. 8. *Eichleriella Schrenkii.* From the type collected at San Antonio, Texas, by H. von Schrenk. *a*, photograph of a piece of limb bearing many fructifications, and *b*, drawing of median longitudinal section of single fructification, × 16.

Fig. 9. *E. Leveilliana.* From specimens collected at San Antonio, Texas, by H. von Schrenk.

Fig. 10. *E. alliciens.* From specimen collected at San Diego de los Baños, Cuba, by Earle and Murrill, 405, in part.

Fig. 11. *E. spinulosa.* From specimen collected at Priest River, Idaho, by J. R. Weir, 55.

Fig. 12. *E. gelatinosa.* From specimens collected in Jamaica by W. A. Murrill and W. Harris. *a*, upper surface of No. 180; *b*, type specimen, 1087, split longitudinally to show thickness of pileus and structure.

Fig. 13. *Sebacina incrustans.* *a*, from specimen collected at Middlebury, Vermont, by E. A. Burt; *b*, from specimen with pileate flaps, collected at Asheville, North Carolina, by H. C. Beardslee, 03126.

Fig. 14. *S. Helvelloides.* From specimen collected at Alcove, New York, by C. L. Shear, 1221. *a* shows upper surface; *b* is a vertical section from the same fructification to show thickness.

Fig. 15. *S. chlorascens.* From type specimen collected at Cocoanut Grove, Florida, by R. Thaxter, 98.

Fig. 16. *S. Shearii.* From type specimens collected at Washington, District of Columbia, by C. L. Shear, 1238.

Fig. 17. *S. calcea.* From specimen on white cedar bark, collected at Middlebury, Vermont, by E. A. Burt.

Fig. 18. *S. cinnamomea.* From type specimen collected at Takoma Park, Maryland, by C. L. Shear, 1339.

Fig. 19. *S. adusta.* From type specimen collected at Priest River, Idaho, by J. R. Weir, 12.

Fig. 20. *S. plumbea.* From type specimen collected at Bingen, Washington, by W. N. Suksdorf, 862.

Fig. 21. *S. atrata.* From specimen collected at Chocorua, New Hampshire, by W. G. Farlow.

BURT—THELEPHORACEAE OF NORTH AMERICA

8. EICHLERIELLA SCHRENKII.—9. E. LEVEILLIANA.—10. E. ALLICIENS.—11. E. SPINULOSA.—12. E. GELATINOSA.—13. SEBACINA INCRUSTANS.—14. S. HELVELLOIDES.—15. S. CHLORASCENS.—16. S. SHEARII.—17. S. CALCEA.—18. S. CINNAMOMEA.—19. S. ADUSTA.—20. S. PLUMBEA.—21. S. ATRATA.

ENZYME ACTION IN THE MARINE ALGAE

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In a previous contribution from this laboratory[1] attention has been called to the difficulties experienced in demonstrating enzyme action in *Fucus vesiculosus*. Because of the negative results there obtained it was deemed worth while to extend the study to certain representative forms of the three great groups of marine algae, the "greens," the "browns," and the "reds"; first, to ascertain whether this apparent inactivity were generally characteristic of the algae, and second, because of the light such an investigation might shed upon the general metabolism of the group.

Historical

Knowledge concerning enzyme activity and the distribution of enzymes in the algae is extremely meagre. The few papers that have found their way into the literature have been, for the most part, by-products of other studies and as such have dealt merely with isolated phases of the subject. From time to time, previous to actual demonstration, the presence of enzymes has been suggested by the work of various investigators. Arber ('01), attacking the problem of carbon assimilation in *Ulva latissima*,[2] found that the accumulation of starch in the tissue disappeared very slowly when the plant was subjected to darkness. This would suggest the presence of a diastase acting slowly. Spargo ('13) observed that *Chlamydomonas* began growth more slowly when the medium contained sucrose as a source of carbon than when dextrose was supplied. She suggests that the sugar is probably assim-

[1] Duggar, B. M. and Davis, A. R. Enzyme action in Fucus vesiculosus. Ann. Mo. Bot. Gard. 1:419–426. 1914.

[2] The binomials used throughout the historical review are those employed by the original investigators, no attempt being made to have them conform to any different existing nomenclature.

ilated in the hexose form and that sucrose must be split by invertase before becoming available. It is a well-known fact that diverse fresh-water algae can be grown in pure culture on media where asparagin and peptone are sources of nitrogen. It is hardly conceivable that the large protein molecule is assimilated directly and, *a priori,* this would argue for the presence of both an ereptase and a desamidizing enzyme.

ENZYMES FOUND IN THE MARINE ALGAE

Few workers have demonstrated enzymes present in either the fresh- or salt-water algae. Fischer ('05), working on the storage carbohydrates of *Anabaena* and *Oscillatoria,* found that the specific carbohydrate involved, which he named anabaenin, disappeared when the algal tissue was autolysed at 40°C. Microchemical tests showed glycogen split off. The action here, if it be due to ferments of the alga, is interesting in that the action was inhibited by .1 per cent acetic acid, by 1 per cent carbolic acid, and still more strangely, by concentrations of ethyl alcohol as low as 5 per cent. One per cent carbolic acid is quite often used as an antiseptic in enzyme experimentation, and the resistance of enzymes to even high concentrations of alcohol is common knowledge. No attempt was made to isolate the enzyme or to carry on experiments outside the cell.

Teodoresco ('12) found that *Chlamydomonas* in pure culture gave rise to an extracellular enzyme that decomposed sodium nucleate with the liberation of phosphorus. Later, ('12[a]) he demonstrated nucleases present in certain "blue-greens," "browns," and "reds." Unfortunately, differences in methods do not permit a true comparison of activity with that of the nuclease isolated by Dox ('10) from *Penicillium camemberti,* nor with that determined by Zaleski ('07) in the growing tips of *Vicia faba,* yet even a crude comparison is interesting. Dox added 2 grams of mold powder to 100 cc. of a 2 per cent solution of yeast nucleic acid, and maintaining his flasks at a temperature of 35–37°C. for forty-five days, found 51 milligrams of phosphorus (calculated as phosphoric acid) liberated. Teodoresco used a .5 per cent solution of sodium

nucleate with an unstated amount of crushed seaweed. The temperature during the incubation period varied from 21 to 26°C. for the different forms used. The following are his results for 100 cc. of substrate:

TABLE I

Alga	Days	Phosphorus as P_2O_5 mgms.
Cladophora frusta	57	54.3
Ceramium rubrum	51	76.6
Griffithsia setacea	37	62.5
Phormidium sp.	15	90.0

Zaleski crushed growing tips of *Vicia faba,* added water and an antiseptic, and allowed this material to autolyse at 34°C. for 4 days. At the end of that time the control flask showed a free phosphorus content of 13.6 milligrams and the one containing the active enzyme 51.2 milligrams. We have no means of knowing even the relative amount of enzyme present in any of these experiments and yet it seems that the algal nuclease compares very favorably with that isolated from the fungi and the higher plants.

The classes in plant physiology at the Marine Biological Laboratory, Woods Hole, for several years past have qualitatively determined diastase in *Ulva lactuca.* Bartholemew ('14), working on the question of starch in the *Florideae,* conclusively demonstrated diastase present in such "reds" as *Polysiphonia variegata, Dasya elegans, Agardhiella tenera,* and *Ceramium sp.* In order to isolate the enzyme, he used the ordinary method of precipitation by alcohol from an aqueous extract of crushed tissue. Starch as paste was hydrolysed rather slowly to an undetermined reducing sugar, presumably dextrose, 5 cc. of .25 per cent starch paste with a relatively large amount of the enzyme material requiring from 6 to 9 days for the completion of hydrolysis. Microscopic observation of the attacked starch grain showed corrosion similar to that caused by the translocation diastase of the barley. Torup (Krefting and Torup, '09) had previously isolated an enzyme from fresh *Laminaria* that hydrolysed the characteristic storage carbohydrate of that alga, laminarin, to dextrose.

Atkins ('14) investigated the oxidases and peroxidases of twenty-nine diverse algae. Using guaiacum as a reagent, oxidases were demonstrated in but one—*Furcellaria fastigiata*—while peroxidases were shown present in seven. Alpha naphthol gave negative reactions for all the forms studied, while with it peroxidases could be determined in but two—*Delesseria sanguinea* and *Furcellaria fastigiata*. He calls attention to the reducing power of the tissues of certain algae and suggests that such agents may be responsible for the failure to obtain positive tests in the other forms. Reed ('15, '15[a]), on the other hand, holds that many of these algae may show a specific oxidative ability. Like Atkins, he found that the ordinary reagents, such as gum guaiac, alpha naphthol, and aloin, gave negative results in all but one or two instances. When, however, alpha naphthol and para-phenylenediamine, para-phenylenediamine alone, or the hydrochlorides of these two were used in the presence of peroxide, positive tests were very generally obtained.

As earlier indicated, the results obtained by Duggar and Davis ('14) for *Fucus vesiculosus* were very generally negative. This was true even though a great variety of substrates were used under varying conditions, and only vigorously growing plants, fresh crushed, or dried and powdered, were employed for enzyme action. The results are exceedingly difficult to explain. It might well be that the enzymes were present but in such small amounts as to escape detection by the ordinary methods. Methods of enzyme isolation are still crude and they undoubtedly involve some loss of the ferments. Another factor suggested in the preliminary paper, was that the death of the cell might liberate certain substances which would then be free to unite with the enzyme complex, throwing it out of the sphere of action.

SOME STORAGE PRODUCTS OF THE ALGAE

It is often assumed that the presence of storage products in the plant is generally linked with the presence of specific enzymes—starch with diastase, inulin with inulase, fats with lipase, hemicelluloses with cytase, etc. These enzymes may be present at all times, as the diastase of the potato tuber and

the diastase and maltase of the barley grain, or they may only arise when there is food transformation and translocation, as in germinating seeds. However, in the light of such possibilities of association, it is worth while to call attention briefly to some of the work that has been done on the chief storage products of the algae.

The carbohydrates have been more worked over in this respect than has any other chemical group, but much confusion still exists regarding their exact status in assimilation. Much of the study has been on the cleavage products, obtained by acid hydrolysis, of undetermined carbohydrates. These, however, are not a true index of the distribution and more restricted chemical nature of assimilable carbohydrates in the living plant; one must look rather to the work of those who have limited themselves to the isolation and determination of unaltered carbohydrates.

CHLOROPHYCEAE

Polysaccharides.—Nägeli ('63) reported "sphärokristalle" in *Acetabularia* which Leitgeb ('87) later showed were inulin. The former worker also demonstrated the presence of this carbohydrate in various members of the *Dasycladaceae.* Küster ('99) has more recently found characteristic crystal formations in *Derbesia* and *Bryopsis* which, from the many reactions they gave, appear to have been inulin. Famintzin ('67) and Krause ('70) worked on the effect of light on starch formation in *Spirogyra,* and within recent years, Timberlake ('01) has contributed observations on the starch of *Hydrodictyon.* Oltmanns ('05, p. 147) speaks of starch accumulation in the *Conjugales, Volvocales, Ulotrichales, Charales, Siphonocladiales,* and some of the *Siphonales.* He considers it the first visible product of assimilation, but thinks that it may also function as a reserve. Starch in the marine forms seems to be quite widely distributed. In the work of Arber ('01), to which reference has already been made, starch accumulation in the tissues of *Ulva, Cladophora,* and *Enteromorpha* was easily demonstrated by means of iodine. Swartz ('11) isolated starch from *Ulva* but was unable to prove its presence in *Enteromorpha,* a closely re-

lated genus. She concluded that the carbohydrates existed in the form of hemicelluloses, probably as pentosans.

Glycogen, although frequently found in the "blue-greens," where, as held by some authors (Fischer, '05), it functions as the chief reserve carbohydrate, has been demonstrated in but one case, as far as is known, in the *Chlorophyceae,* and that by Beyerinck ('04) in *Chlorella variegata.*

Simple sugars.—The nature of the simple sugars in the group is indefinite. Klebs ('96) reported a substance in the cells of certain *Heterokonteae* that reduced Fehling's solution, but this means little since most algae contain non-carbohydrate reducing substances made up chiefly of tannins and tannoidal bodies. Tihomirov ('10) used the phenylhydrazine method as modified by Senft ('04) for the detection of osozone-forming sugars in algal tissues in this group, chiefly those of *Codium bursa* and *C. tomentosum.* After a period of thirty days, for these two forms, yellow amorphous deposits appeared in the cells indicating a sugar reaction. The definite sugars these osozones represented could not be determined, but he suggests the possibility of dextrose and d-galactose. It seems evident that they must be present in very small quantities in the tissues investigated.

PHAEOPHYCEAE

Polysaccharides.—Starch is conspicuously absent from the great group of "browns," but there are, however, certain less highly condensed polysaccharides present. Schmiedeberg ('85) speaks of a dextrin-like compound which he isolated from *Laminaria.* He gave to it the name "laminarin" and the general formula, $10(C_6O_{10}O_5)$-$9H_2O$. There seems, however, to be some confusion regarding his method of arriving at these figures. Torup ('09) was able to extract a dextrin from *Laminaria sp.* with warm water, that gave dextrose on hydrolysis. This could be isolated only during the winter months. He called it "kreftin." Kylin ('13), extracting crushed *Laminaria saccharina, Fucus vesiculosus,* and *Ascophyllum nodosum,* obtained a dextrin-like compound similar to that described by Schmiedeberg and he retained Schmiede-

berg's name, "laminarin." He showed also that Torup's "kreftin" was without doubt a modification of "laminarin." Kylin ascribes to "laminarin" the same physiological function that starch performs in the higher plants, i. e., that of a reserve product. In a more recent paper ('15) he shows that there is an accumulation of the "laminarin" in the tissues of the algae during the summer months, while during the winter and spring this reserve is drawn upon by the young fronds until by the end of March very little of it is demonstrable.

Kylin was also able to clear up much of the confusion that has attended observation of the light-refracting granules present in the cells of many members of the group. They had been variously considered as of fatty nature, proteinaceous, tannin-like, and glucosidal. Reinke ('76) demonstrated fat-like bodies in the cells of *Fucus* that he looked upon as the first visible products of assimilation, a point of view later supported by Hansen ('93). Schmitz ('83) claimed two distinct bodies present, one of which, although it did not react with iodine, he called "phaeophyceenstärke," the other giving the ordinary reactions for fats. Hansteen ('92) had observed bodies in the same plant which he maintained were of carbohydrate composition and to which he applied the term, "fucosankörner." Crato ('92, '93), the same year, investigating the fat globules observed by Schmitz, suggested that they were either phloroglucin or a derivative of it, since they colored red with vanillin-hydrochloric acid. This conception was held by Bruns ('94) as well. In a later paper, Hansteen ('00) observed that the "fucosankörner" were formed in the presence of light, and this to his mind indicated that they function as the first assimilable products. Hunger's ('02) work two years later pointed to Hansteen's "fucosankörner" as being glucosidal in nature, the carbohydrate attached being bound up with phloroglucin, or at times, with tannic acid. Some of the larger "körner" gave fat reactions, some protein. Kylin found three definite bodies in the cell, the nature of which had been confused by earlier workers—fat globules, proteinaceous particles, and tannin-like bodies—these latter probably representing the "fucosankörner" of

Hansteen. He holds that none of these are to be considered the first visible products of assimilation, and suggests that here, as in most phanerogams, carbohydrates function in that rôle.

Simple sugars.—As far as is known, Tihomirov ('10) was the first to definitely demonstrate simple sugars in these plants. He used the same phenylhydrazine method employed with the "greens," but as was the case there, was unable to connect the osozones with definite sugars. The osozones took considerable periods of time to form, in some cases as long as five months, evidence pointing to the low concentration of sugars in the cell. It is a question, too, whether during this long period of incubation some of the more highly condensed carbohydrates in the cell were not hydrolysed far enough to give the sugar tests. Using the same method, Kylin ('13) was unable to substantiate these results. However, by using 40 per cent alcohol as an extracting agent, precipitating the inorganic material with lead acetate, and then purifying with alcohol, he was able to obtain reducing sugars from several of the *Fucoideae,* particularly *Laminaria digitata, L. saccharina, Ascophyllum nodosum,* and *Fucus vesiculosus.* In all cases Seliwanoff's test for fructose was positive, while dextrose was demonstrated by its osozone. These sugars he considers the first products of assimilation referred to above.

RHODOPHYCEAE

Polysaccharides.—The so-called Florideae-starch has been the source of many investigations, from the time of Nägeli ('58) and Van Tieghem ('65) to the present day. Although not identical perhaps, it is very similar to the starch of the higher plants, and as very generally held, it undoubtedly functions in the same manner. Meyer ('95), Kolkwitz ('00), and Bartholemew ('14) hold the opinion that it represents a combination between true starch and dextrin, while Bütschli ('03) suggests the possibility of its being a transitional stage between amyloporphyrin and amyloerythrin. Kylin ('13) considers it as standing midway between starch and dextrin. This investigator succeeded in isolating

Florideae-starch from *Furcellaria fastigiata,* that was readily hydrolysed to dextrose by malt diastase, and it will be remembered that Bartholemew ('14) isolated diastase from several of the "reds" that split phanerogamic starch to reducing sugars.

Simple sugars.—Very little work has been done on the di- and monosaccharides of the "reds." Tihomirov ('10) succeeded in obtaining the same yellow amorphous osozone deposits in the tissues of *Sphaerococcus crispus* and *Gigartina mamillosa* that he had in certain members of the "greens" and "browns," but here, as in the other groups, the specific osozone involved could not be determined.

FATS AS STORAGE PRODUCTS

Many observations have made it evident that fats in some form or other are generally present in the algae, their peculiar rôle, however, having been very little investigated. In some of the siphonaceous forms, particularly *Vaucheria,* they seem to replace carbohydrates. Whether fats are to be regarded as the first visible products of assimilation in these forms is disputed. Some workers hold them to be reserve products, some by-products of metabolism. If they are utilized as a reserve or storage product in any of the forms, one might expect to find evidences of lipolytic action, yet none has been reported so far.

As stated by Czapek ('13, p. 761), Loew and Bokorny find that *Spirogyra* and other filamentous forms contain 6 to 9 per cent of the dry weight as fat. This probably includes lecithin. The same authority gives the following results as obtained by Sestini, the figures being percentages of the dry weight:

Vaucheria pilus	2.94
Ulva latissima	.21
Fucus vesiculosus	.67
Valonia aegagropila	.15
Gracilaria confervoides	.11

König and Bettels ('05) made a large number of analyses of the dry tissues of a variety of marine algae and found a fat

content ranging from .20 per cent in *Enteromorpha* to .98 per cent in *Porphyra.*

RELATION OF THE ALGAE TO NITROGEN

Some of the recent work on pure culture methods with fresh-water algae, such as that of Beyerinck ('90), Charpentier ('03, '03[a]), Chick ('03), Artari ('13), Spargo ('13), and Schramm ('14) have conclusively proved that these forms can utilize organic nitrogen. Furthermore, the work of Letts and Hawthorne ('11), and Foster ('14) point to the fact that the marine forms may have this capacity as well. Letts and Hawthorne and also Letts and Richards ('11) showed that *Ulva latissima* grew better in sewage-contaminated sea-water than in water from the open sea. Foster placed strips of *Ulva lactuca* in normal and artificial sea-water, containing in addition compounds of nitrogen in varying concentrations. When urea or ammonium sulphate was added to either solution an accelerated growth took place.

The current conception concerning the assimilation of organic nitrogen by the animal organism is that the protein and amino acid molecule must be completely desamidized before the building-up process can begin. In the absence of definite information to the contrary, we can conceive of a parallel situation existing in the plant. The question at once arises in regard to the algae, whether this be due to the agency of amidases formed by the tissue, or to the activity of desamidizing bacteria, the presence of which Brandt ('99), Gran ('02), Baur ('02), Reinke ('03), Benecke and Keutner ('03), and others have shown to exist abundantly in harbor waters. Neither Letts and Hawthorne nor Foster worked with pure cultures, and these bacteria may have been the agency in their experiments to render the amino-nitrogen assimilable.

CARBOHYDRATES AND CARBOHYDRATE CLEAVAGE PRODUCTS OF ALGAL SLIME

Besides the carbohydrates that may be directly assimilable, we find those whose function in metabolism is more or less disputed. The so-called algal slime is made up chiefly of such products.

Chemical composition.—Greenish ('81) found agar from *Fucus amylaceus* to consist of 37.21 per cent gelose (probably galactan since it passed to galactose on hydrolysis) and that from *Sphaerococcus crispus* of 60 per cent of the same carbohydrate. König and Bettels ('05) give the carbohydrate composition of agar-agar from *Gelidium* as 33 per cent galactans and 3.1 per cent pentosans; by hydrolysis, d-galactose and levulinic acid were split off. Günther and Tollens ('90) found fucosan in *Fucus* from which the methyl-pentose, fucose, was split off. Galactose was also demonstrated. Sebor ('00) obtained galactose, glucose, and fructose from the slime of *Chondrus crispus* by acid hydrolysis. He held that the slime is a very complex carbohydrate of high molecular weight, made up chiefly of galactosan, glucosan, and fructosan.

The cleavage products of *Porphyra laciniata,* as investigated by Oshima and Tollens ('01), were found to consist chiefly of l-galactose and mannose, but glucose, fucose, and other pentoses were also obtained. Müther and Tollens ('04) found methyl-pentosans in several of the *Fucaceae.* König and Bettels ('05), working on the carbohydrate hydrolytic products of various species of *Porphyra, Gelidium, Laminaria, Cystophyllum,* and *Enteromorpha,* found them to consist of such hexoses as galactose, dextrose, and fructose, as well as several pentoses, chiefly methyl-pentoses. *Enteromorpha* yielded a pentose—rhamnose. The results of Swartz ('11) agree with those above, namely, that for all forms studied, representatives of the "greens," "browns," and "reds," pentosans were always present, and galactans frequently so. Kylin ('13), by direct extraction with warm water of crushed *Ceramium, Furcellaria,* and *Dumotia,* obtained substances that gave the mucic acid test for galactose, as well as the phloroglucin test for pentosans. Substances giving pentosan reactions alone were isolated from the slime of *Ascophyllum nodosum, Fucus vesiculosus,* and *Laminaria sp.* He was apparently unable to substantiate the finding of galactan in *Fucus* by Günther and Tollens, and this negative result also conflicts with the statement of Swartz, who says that the gelatinization in the algae is due to the galactan groups.

Kylin ('14) and others have also demonstrated pectin-like compounds forming the middle lamella in various members of the *Fucaceae*. These exist as the calcium salts of pectic-like acids which Kylin designates "Fucinsäure" and "Alginsäure."

PHYSIOLOGICAL SIGNIFICANCE OF ALGAL SLIME

It is seen that algal slime is made up chiefly of the anhydrides of hexoses and pentoses—carbohydrates that must be broken down to simpler form before assimilation by the plant would be possible. Two questions naturally arise: (1) Do the algae concerned form enzymes that will hydrolyse these highly condensed carbohydrates to assimilable form? (2) Does the slime itself arise through the breaking down of the hemicelluloses of the cell wall through enzymic or other causes, or does it represent a final stage in the condensation of those hemicelluloses?

Algal slime as a reserve product.—Galactanases and mannases have been demonstrated in the phanerogams and in the fungi by Bourquelot and Hérissey ('99), Grüss ('02), and Hérissey ('03). The last worker especially has clearly shown the distinct rôle that galactans and mannans may play as reserve products in the tubers of the *Orchidaceae* and in many of the *Leguminoseae*. It is significant that Gran ('02[a]) was able to isolate a marine bacillus, *B. gelaticus*, that acted on part of the constituents of agar-agar to give a reducing sugar. From the standpoint of a possible symbiosis it would be interesting to know if this organism has the ability to fix free nitrogen. Saiki ('06) experimented with a number of algal and lichen preparations containing a large proportion of carbohydrates as galactans and pentosans, and concluded that the latter could not be transformed into sugars readily by carbohydrate digesting enzymes of animal origin and scarcely more so by the vegetable enzymes, either of the higher plants or of bacteria.

Still less is known of the digestion of pentosans by the higher plants. Schöne and Tollens ('92) found no decrease in the amount of pentosans during germination and conclude

that they cannot function as reserves. Cross, Bevan, and Smith ('95) consider the pentosans as by-products of metabolism and once formed remain unalterable. Ravenna and Cereser ('09), on the other hand, in some very interesting experiments, found that when dextrose was supplied as the sole nutrient to the leaves, pentosans increased greatly, especially in the light. If, however, the function of chlorophyll is inhibited, a decrease in the amount of pentosans takes place. These results form the basis for their conclusion that pentosans may sometimes function as reserves.

The origin of algal slime.—The question concerning the origin of the slimy and gummy constituents of cells, whether they arise through enzyme action or through other causes, has provoked much discussion. There is considerable doubt whether such gums can arise directly from true cellulose or whether they are, at least in the case of the plant mucilages, laid down as such.

One might roughly group the plant gums into those arising as a result of some external excitant, such as, for example, cherry gum, acacia gum, gums of citrus, etc., and those which seem to be normal constituents of the plant, as the mucilages found in the epidermis of many seeds and plant organs. The former arise as a result of a pathological condition; the latter, as far as we know, are normal physiological products and as such are more nearly comparable to the algal slime.

Klebs ('84), investigating slime formation in some of the lower algae, particularly some of the *Desmidiaceae,* held that it was not a conversion product of cellulose. Hauptfleisch ('88) substantiated the conclusion of Klebs, and going further, states that it arises in this particular case through the activity of the protoplasm, being excreted through pores. Oltmanns ('04, p. 76) illustrates very clearly the arrangement of these pores. Tschirsch ('89) differentiates these slimes or mucilages into those giving a cellulose reaction and those not doing so, the former having some relation perhaps to the cellulose, but the latter being laid down on the cell wall as such by the protoplasm. He holds the epidermal slime of *Spirogyra* to be of this latter type, which he calls "echter Schleim." In

the same work the author concludes the slime of the *Fucaceae* and of the *Florideae* to be of the "echter" type, occurring here, however, not as a layer laid down on the inner cell wall, but as an intercellular substance. Guignard ('93) held much the same view, and in an excellent histological investigation, clearly demonstrated the presence of slime or mucilage ducts in the *Laminariaceae*.

Mucilages very similar in nature and origin to the algal slimes occur in the higher plants, and much more work has been done with them than with those occurring in the algae. It is hardly necessary to go into the historical aspect of this phase of the work. The current conception of its origin is voiced by Walliczek ('93), who, investigating rather fully the location of different types of normal mucilages by means of suitable stains, found that in almost all cases they were laid down as such. According to him, the slime forms secondary layers on the cell wall which he designates "Membranverdickungsschichten"—layers that in many instances almost completely fill the cell. Where the epidermal layer of seeds becomes gelatinous, as, for example, in those of flax, mistletoe, various *Cruciferae*, etc., it is this inner cell wall which Walliczek holds to be the seat of slime formation. Upon contact with water the slime swells remarkably, filling the cell and at times even bursting it. There may or may not be an actual hydrolysis of the true cellulose, but if there is it seems rarely to enter into mucilage formation.

Experimental

Forms used.—The algae to be used for enzyme investigation were collected in the vicinity of Woods Hole, Massachusetts, during the summers of 1913–14, at which time the plants were also dried for winter work at the Missouri Botanical Garden. Work with the fresh tissue was carried on at the Marine Biological Laboratory, Woods Hole, during the latter summer. The selection of forms with which to work was limited to those relatively abundant in the neighboring waters, a further limiting factor in selection being relative freedom from adhering marine organisms. Only those plants

were selected that were "clean." This was an important precaution, since many adhering organisms have been found to be quite active enzymatically, and the presence of even a few might well lead to serious errors in the final results. The following forms lent themselves most readily to the work:[1]

Chlorophyceae
Ulva lactuca (L.) Le Jolis
Enteromorpha intestinalis (L.) Link

Phaeophyceae
Laminaria Agardhii Kjellm.
Ascophyllum nodosum (L.) Le Jolis
Mesogloea divaricata (Ag.) Kutz

Rhodophyceae
Ceramium rubrum (Huds.) Ag.
Agardhiella tenera (J. Ag.) Schmitz
Rhodymenia palmata (L.) Grev.
Chondrus crispus (L.) Stack.

Preparation of algal material.—In addition to the question of cleanliness, great care was taken to select only plants that were in a young, vigorously growing condition. These were brought into the laboratory, placed in large aquarium jars containing salt water, picked over, and all detectable foreign matter removed. A thorough washing in running salt water for two hours was then given, after which, with the exception of one or two forms that rapidly gelatinized, the plants were placed in running fresh water for 10 or 15 minutes. This fresh water treatment was very efficacious in causing small snails and other minute marine organisms to loosen their hold.

The plants so washed were either crushed and used at once with the substrate for enzyme action, or they were dried for future use. In either case, two general ways of using the ma-

[1] With the exception of *Laminaria Agardhii* and *Agardhiella tenera*, these binomials conform to the nomenclature as given by Farlow (Marine algae of New England, pp. 1–210. *pl. 1–14.* 1881); these two forms are as given by De Toni (Sylloge Algarum 3: p. 349. 1895) and Engler and Prantl (Nat. Pflanzenfam. 1^2:371. 1896), respectively.

terial for such action were employed. The tissue was added directly to the substrate, or it was extracted with water by the method to be described later and a water-diffusion used of the alcohol precipitate. If the fresh tissue were to be used directly, it was ground in a meat chopper two or three times, then pounded in a large mortar with an equal amount of fine, clean, quartz sand. This treatment gave a very homogeneous pulp, one in which a large number of the cells were broken down. If desired for future use, the plants were either dried at room temperature or dehydrated by the following modified Buchner "dauerhefe" process:

3 volumes	95 per cent alcohol	for 15 minutes.
3 volumes	acetone	for 15 minutes.
3 volumes	95 per cent alcohol	for 10 minutes.
3 volumes	acetone	for 5 minutes.
2 volumes	absolute alcohol or ether	for 5 minutes.

After each treatment, the dehydrating liquid was pressed out through two thicknesses of cheese cloth by making a tourniquet. Upon the removal of the absolute alcohol or ether, the tissue was spread out on adsorbent paper, either filter paper or paper toweling, until all the dehydrating agent had evaporated. A uniformly dry, brittle, easily crushed material usually resulted that was roughly broken up and stored in tightly stoppered bottles for future use. Those plants that were dried at room temperature were simply wrapped in paper or placed in paper bags until needed.

The crushing of the dry material was accomplished in the same manner as was the fresh. Usually it was ground twice or more in an ordinary meal mill, then pounded in a mortar with an equal weight of quartz sand until a very fine powder was obtained. The sand was dispensed with if the tissue were easily crushed.

Methods of isolating the enzymes.—As indicated above, there were two general methods of using the material for enzyme action: first, adding the crushed tissue directly to the substrate, either as fresh pulp or as "dauerhefe" powder;

second, by extracting the tissue with water and precipitating the protein-enzyme complex with several volumes of 95 per cent alcohol. Wherever possible the first method was used, since it was thought that in this way the maximum enzymic activity would be obtained. However, the fresh pulp and the powdered material contained a substance, or substances (probably tannoidal bodies), that reduced copper from Fehling's solution, and so in all experiments where sugar determinations were involved, it was found necessary to use the extraction and precipitation method; by this means all the unknown reducing substances were avoided. The method was as follows:

To a known amount of the crushed, fresh algal material, 3–5 volumes by weight of distilled water were added; to the powdered tissue, 8–10 volumes. The amounts varied owing to the differences in viscosity produced by the different algae. In some forms a relatively large amount of water was necessary in order to overcome difficulties in handling due to this high viscosity. Two per cent toluene was generally added as an antiseptic, or in some cases, 1 per cent chloroform-thymol mixture was used (5 per cent thymol dissolved in chloroform), and the extraction allowed to go for 12 hours at room temperature, or for 4 hours at 35° C. The water extract, if at all viscous, was then filtered off through two thicknesses of cheese cloth and the algal tissue pressed out as completely as possible by making a tourniquet of the cloth. Filtering through cotton was tried at first, both with pressure and without, but the method had the disadvantage of slowness and also that of adsorption by the cotton. Neither did filter paper lend itself efficiently to the filtration of such viscous liquids, a drier residue being obtainable in a shorter time by the cheese cloth-tourniquet method. A press would have been desirable but none was at hand. If the medium were not viscous, it was filtered with pressure through a thin layer of cotton or a coarse filter paper in the bottom of a Buchner funnel.

The protein-enzyme complex was precipitated with 3 volumes of 95 per cent alcohol. After a few moments the

coagulum either came to the top or settled to the bottom of the vessel—if to the top, it was usually very much aggregated and little difficulty was experienced in the filtering, if to the bottom, it was generally in a very finely divided condition and unless care was exercised in the decantation of the supernatant liquid the pores of the filter soon became clogged, resulting in extremely slow filtration. Time was therefore given for a complete settling out (15 minutes to half an hour sufficed) and all the clear fluid filtered off before the coagulum reached the filter paper.

A homogeneous diffusion of the precipitate was made by placing the filter paper with the attached coagulum in a known volume of distilled water. The paper could soon be removed without loss of material, and the weight of the original fresh or dry tissue represented by an aliquot portion of the solution easily reckoned. If the precipitate were not required immediately, it was dried on a filter paper at room temperature and stored in stoppered jars. In none of the experiments was the enzyme material purified further.

When dissolved in water, the precipitates behaved differently. Some, especially those where much slime had been noticed in the extraction, gave an extremely viscous suspension, others a suspension of low viscosity. In *Laminaria* and *Chondrus,* where the extract had been quite viscous and slimy, the protein was caught up in the precipitated slime in such a way as to make the freeing of it practically impossible. The precipitate in these cases was very large and when diffused in water gave a suspension difficult to handle. *Rhodymenia, Ceramium,* and *Enteromorpha,* on the other hand, gave a finely divided precipitate that produced no viscosity.

Glassware, antiseptics, solutions, etc.—With few exceptions, the various experiments were set up in 125 cc. Erlenmeyer flasks. All glassware was thoroughly cleaned with strong soap and then with chromic-sulphuric cleaning mixture, after which it was rinsed several times with tap and distilled water.

Solutions were made up from either Merck's or Kahlbaum's "guarantiert" chemicals.

Three general antiseptics were used—toluene, alcohol to 20 per cent, and 5 per cent thymol in chloroform. Toluene was, in general, the most satisfactory. Usually it was used to 2 per cent concentration, but where large surfaces were exposed, as high as 4 per cent was found necessary. The chloroform-thymol was also very efficacious, but in the carbohydrate experiments chloroform could not be used because of its power of reducing copper. In the lipase work the substrate was made up to 20 per cent alcohol since the action seemed to proceed best in the presence of this antiseptic. In all cases where the experiments were maintained over a considerable period of time, it was necessary to add additional antiseptic from time to time.

Checks were set up in all experiments—on the substrate, on the material used to demonstrate enzyme action, and on the substrate plus such enzyme material boiled to destroy any ferments that might be present.

CARBOHYDRASES OF THE ALGAE

In these experiments the alcohol precipitate from an aqueous extract of crushed, fresh or dried, algal tissue was employed as an enzyme source, this precipitate being diffused in such a volume of distilled water that one gram of the original material was represented by 5 cc. of the diffusion. Thus one can more closely compare the amounts of enzyme present in definite amounts of different algal tissue. The number of cubic centimeters of diffusion will be noted in connection with each set of experiments.

Substrates.—Starch, dextrin, inulin, sucrose, maltose, lactose, glycogen, and in one or two cases, laminarin isolated from *Laminaria Agardhii,* were used as substrates. These were made up in 1 per cent concentrations with the exceptions of maltose and glycogen, where .25 per cent, and laminarin, where .5 per cent concentrations were employed.

Of the many suggested methods for making up starch paste, the following one used by Clark ('11) was found to give the best satisfaction. Ten grams of potato starch were weighed out and placed in a beaker with 250–300 cc. of distilled water.

This was brought to a boil with constant stirring, and when an opalescent solution resulted the paste was transferred with rinsing to a 2-liter flask containing about 500 cc. of boiling water. The lot was boiled under a reflux condenser for two hours, cooled, and made up to a liter. Although, as is stated by Clark, this treatment is very effective in breaking down the starch grain physically, no detectable hydrolysis takes place, and the additional advantage is gained in obtaining a paste that will not settle out, even after long standing. Two per cent toluene was employed as an antiseptic if the starch were not to be used immediately.

Since all dextrin obtainable contained some reducing sugar, it was found necessary to purify it by making a concentrated solution in hot distilled water, and then precipitating out with several volumes of 95 per cent alcohol. The dextrin was caught on a filter paper and dried at a low constant temperature.

Laminarin, a dextrin-like carbohydrate found in many of the *Fucaceae,* was isolated from *Laminaria Agardhii* according to the method employed by Kylin ('13), with some few slight modifications. Freshly collected *Laminaria* was crushed in the usual way and 1,680 grams of the pulp were boiled with 7 liters of water for 24 hours, water being added from time to time to replace that lost through evaporation. The extract was then filtered off through a double thickness of cheese cloth, and the residue pressed out with a tourniquet. About 3,000 cc. of a dirty brown filtrate were obtained which was divided into three lots of 1,000 cc. each. To the first of these was added a concentrated $Ba(OH)_2$ solution until the precipitation of the inorganic matter was complete. The precipitate was caught on a cotton filter in a Buchner funnel, the filtrate being a clear, golden-colored liquid. The inorganic material in the other two lots was precipitated with basic lead acetate, the liquid filtered off through cotton, and the excess of lead removed with H_2S. The solutions were filtered while hot through double filter paper to remove the lead sulphide, and then the excess of H_2S was driven off with heat. The three portions were first evaporated to about one-

third their volume, when the scum that formed was filtered off; this filtrate was then further evaporated to about one-fifth the original volume on the water bath. At this point the two lead acetate portions were placed together. Ninety-five per cent alcohol was added to each of the lots to about 80 per cent concentration when a flocculent precipitate came down rather slowly. With the $Ba(OH)_2$ portion this was copious, with the lead acetate, slight. After two hours the precipitates were filtered off, washed with absolute alcohol, redissolved in a small amount of distilled water, and then reprecipitated with 4 volumes of absolute alcohol, the resulting precipitate being dried over $CaCl_2$. From the $Ba(OH)_2$ portion, 4.2 grams of a creamy white powder were obtained that gave a very slightly reddish tinge with iodine, did not reduce Fehling's, and was easily soluble in water, giving a clear solution. Upon hydrolysis with weak H_2SO_4 a reducing sugar was split off. The lead acetate portion gave but two grams of the same material. This powder was taken to be the laminarin described by Kylin.

The determination of reducing sugars.—The reduction of copper, or in the case of maltose and lactose, the increase in the reducing value of the substrate plus the enzyme over that of the checks, was taken as the measure of carbohydrate hydrolysis. In this determination the permanganate titration method, as modified and described by Shaffer ('14), was used, it being possible with it to determine amounts of sugars as low as 2 milligrams[1] very accurately and quickly. Shaffer's description may not be generally available to plant workers who may desire to use this really splendid method, and so the various steps in the process as used here are set down in some detail.

Ten cc. of the carbohydrate-enzyme substrate were placed in a large test-tube containing 5 cc. of water, and just brought to a boil. At this point a drop of 50 per cent acetic acid was added. When the slight protein precipitate formed, 5 cc. of

[1] Shaffer determines values below two milligrams, but as used here, consistent results could not be obtained where less than that amount was involved. Below this point the relative increase in the experimental error is large.

colloidal iron (Iron dialysed, Merck) were pipetted in and the tube well shaken, the iron then being flocked out with .25 gram of Na_2SO_4. Upon the addition of this latter the mixture was again thoroughly shaken and the iron precipitate thrown down by centrifuging, the resulting clear, supernatant liquid then being decanted off through a small filter. This filtrate was entirely free of proteins or other substances which, through oxidation later, would lead to errors in the permanganate values. Ten cc. of this filtrate were placed in a 50 cc. lipped centrifuge tube, and standard Fehling's solution added, the copper content of which was in excess of that reducible by the sugar present.[1] The tube was then placed in a boiling water bath for 10 minutes, at the end of which time it was centrifuged at a moderate speed for 2 minutes, the supernatant unreduced Fehling's carefully decanted off, a like volume of distilled water added, and the cuprous oxide again thrown down by a 2-minute centrifuging. All but 1 or 2 cc. of this wash water was carefully decanted off, and the copper dissolved in the smallest amount necessary of a mixture of equal parts of 10 per cent ammonium ferric sulphate and 50 per cent sulphuric acid. It was found that if the copper were stirred up with a glass rod just before dissolving, it went into solution more readily. The dissolved copper was titrated directly in the centrifuge tube against N 50 $KMnO_4$.[2]

By calculation it is found that 1 cc. of N 50 $KMnO_4$ is equivalent to 1.27 milligrams of copper, and for the conversion of this into glucose use was made of the table prepared by Shaffer.[3]

As stated by Shaffer, care must be observed on the three following points: (1) to eliminate all oxidizable substances other than sugar, (2) to titrate the cuprous oxide immediately after dissolving, (3) to use poor conductors of heat as containers of the centrifuge tubes in the water bath, else many broken tubes will result. As employed here, circular wire

[1] In the determinations made here this amount never exceeded 10 cc.

[2] It is necessary to titrate immediately after dissolving because of the danger of oxidation of the cuprous oxide. If larger amounts of sugar are concerned, N/10 $KMnO_4$ may be used.

baskets having wooden bottoms and tops were used, the tops containing holes large enough for the free insertion of the centrifuge tubes, and the bottoms, slight depressions into which the tubes might rest. It is always necessary to run blanks with Fehling's solution since some reduction always takes place. The cuprous oxide solvent must be free from ferrous iron, and this can be assured by the addition of a trace of permanganate.

Method of setting up experiments.—Fifty cc. of the substrate to be used were placed in 125 cc. Erlenmeyer flasks with 2 per cent toluene as an antiseptic. If the series were maintained longer than six weeks, another 2 per cent toluene was added. As previously noted, in these carbohydrate experiments the material used for enzyme action was an alcohol precipitate from a water extract of algal powder or pulp. This was diffused in water so that 10 cc. of the diffusion represented 2 grams of the original tissue. Usually this amount was added to the substrate to be tested. Duplicates and checks were set up in accordance with the following model series for starch:

1. 50 cc. starch, 10 cc. enzyme diffusion.
2. 50 cc. starch, 10 cc. enzyme diffusion.
3. 50 cc. starch, 10 cc. boiled enzyme diffusion.
4. 50 cc. starch, 10 cc. boiled enzyme diffusion.
5. 50 cc. starch, 10 cc. distilled water.
6. 50 cc. starch, 10 cc. distilled water.

[3] To make this table more generally available, it is printed here in full.

Shaffer's table of copper-glucose equivalents

mgms. copper	mgms. glucose	mgms. copper	mgms. glucose	mgms. copper	mgms. glucose
0.7	.47	6.0	2.74	20.0	9.71
1.0	.62	7.0	3.21	25.0	12.25
1.5	.88	8.0	3.68	30.0	14.80
2.0	1.11	9.0	4.15	35.0	17.40
2.5	1.32	10.0	4.65	40.0	20.00
3.0	1.50	12.0	5.61	50.0	25.00
3.5	1.67	14.0	6.61	60.0	30.10
4.0	1.82	16.0	7.61	80.0	40.40
5.0	2.27	18.0	8.65	100.0	50.70

In addition, at the end of a complete carbohydrate series there were included for each alga the following checks:

1. 50 cc. distilled water, 10 cc. enzyme diffusion.
2. 50 cc. distilled water, 10 cc. enzyme diffusion.
3. 50 cc. distilled water, 10 cc. boiled enzyme diffusion.
4. 50 cc. distilled water, 10 cc. boiled enzyme diffusion.

Where the enzyme diffusion referred to above actually contained carbohydrases, it was extremely difficult to render them inactive by heating—10 minutes at the boiling point not being sufficient in most cases to more than slow down the action. This was probably due to the impurities contained, the relatively large amounts of protein and slime present tending to protect the enzymes. Those extracts relatively richer in such constituents proved the more difficult to render inactive. The expedient was finally adopted of placing the enzyme material in the autoclave and bringing the pressure in the latter up to 15 pounds. This proved quite effective.

THE CARBOHYDRASES OF ULVA LACTUCA

The effect of an extract of Ulva lactuca on different starches. —Since starches were to be used in many of the following

TABLE II

THE ACTION OF ULVA LACTUCA "DIFFUSION-EXTRACT"* UPON CERTAIN STARCHES

Starch 50 cc. 1 per cent	15 days		30 days	
	Sugar as glucose in 5 cc.† mgms.	Iodine test	Sugar as glucose in 5 cc. mgms.	Iodine test
Potato	10.1	Blue, trace red	17.4	Complete hydrolysis
Arrowroot	9.8	Blue, trace red	16.9	Complete hydrolysis
Wheat	9.9	Blue, trace red	17.1	Complete hydrolysis
Corn	6.3	Blue	10.8	Reddish purple
Soluble	8.7	Blue	15.9	Traces dextrin
Inulin	....	.....	Trace‡	.....

* Wherever the term "diffusion-extract" is employed, it refers to a diffusion in water of the alcohol precipitate from an aqueous extract of the alga under discussion.

† The sugar values in this and the following tables are net, i. e., sugar values for all checks have been deducted.

‡ In all the following experiments an amount of sugar below 2 mgms. is designated a "trace."

experiments, it was desired to know which, if any, were the most favorable substrates for the diastases of the algae. The action of diastase from *Ulva lactuca* was taken as an index. Potato, arrowroot, wheat, corn, and soluble starch, as well as inulin, were made up in 1 per cent concentrations in the manner previously described. To 50 cc. of each of these substrates were added 10 cc. of a diffusion of an alcohol precipitate from a water extract of dehydrated *Ulva lactuca*. Two per cent toluene was added as an antiseptic, and the flasks maintained at a temperature of 35°C. for 30 days. The results of the experiments are given in table II.

The data show but slight differences in the rate of digestion of the starches with the exception of corn starch, and the reason for this is not clear. One would expect it to be due to some impurity in the starch rather than to an inherent difference in the granule. The action on inulin was so slight as not to warrant the assumption of hydrolysis due to inulase.

The action of an extract of Ulva lactuca upon various carbohydrates.—A series was arranged using a "diffusion-extract" from *Ulva lactuca* with the following substrates: potato starch, dextrin, glycogen, sucrose, maltose, and lactose. Ten cc. of the "diffusion-extract" were added to each flask with 50 cc. of substrate, 2 per cent toluene used as an antiseptic, and the flasks maintained at a temperature of 35°C. for 30 days. The data are given in table III.

TABLE III

THE ACTION OF AN EXTRACT OF ULVA LACTUCA UPON VARIOUS CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.	
	15 days	30 days
Starch	10.20	15.50
Dextrin	6.30	9.95
Glycogen	2.25	3.50
Sucrose	.....	Trace
Lactose	.....	.....
Maltose	.....	Trace

The two polysaccharides, starch and dextrin, are very readily attacked even though the action is slow. Glycogen, which is hydrolysed by most diastatic enzymes with about the

same ease as starch, seems very slightly acted upon by the carbohydrases of *Ulva*. The failure of action on sucrose and lactose is not so surprising as is that on maltose, for one would expect the action on polysaccharides to continue to what is generally held to be directly assimilable sugars, i. e., the hexoses.

THE CARBOHYDRASES OF ENTEROMORPHA INTESTINALIS

This series (table IV) was run under exactly the same conditions as the one preceding. The "diffusion-extract" was from dehydrated tissue about two months old. Ten cc. of this were used with each 50 cc. of substrate, toluene added as an antiseptic, and the flasks kept at a temperature of 35° C. for 30 days.

TABLE IV

THE ACTION OF A "DIFFUSION-EXTRACT" FROM AIR-DRIED ENTEROMORPHA TISSUE UPON CERTAIN CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.	
	15 days	30 days
Starch	9.7	13.1
Dextrin	5.1	9.8
Glycogen	2.8	3.9
Inulin	Trace	Trace
Sucrose	....	Trace
Lactose	....	....
Maltose	....	....

The results for this closely related form are consistent with those obtained for *Ulva*, the action in the present case, however, being somewhat slower. The more common polysaccharides are acted upon while the disaccharides are not attacked.

THE CARBOHYDRASES OF LAMINARIA AGARDHII

The water extract from air-dried Laminaria tissue was extremely viscous and upon addition of alcohol, a very heavy precipitate was thrown down that contained a large amount of algal slime. When water was added to this precipitate in the usual ratio a very viscous diffusion was obtained. Ten cc. of the "diffusion-extract" were used with 50 cc. of the substrate and 2 per cent toluene added as an antiseptic. The flasks were kept at a temperature of 20–22° C. for 100 days,

portions being removed and sugar determinations made at the definite intervals noted in table v.

The carbohydrases in this form appear to be limited to those acting on starch and dextrin, and with these the hydrolysis proceeds much more slowly than was true with either of the preceding "greens." The lower temperature at which the hydrolysis occurred does not explain completely the lessened action. Inhibiting substances or else an actually smaller concentration of the enzyme seem to be important factors.

TABLE V

THE ACTION OF A "DIFFUSION-EXTRACT" FROM AIR-DRIED LAMINARIA TISSUE UPON CERTAIN CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.			
	15 days	45 days	75 days	100 days
Starch	Trace	3.25	4.7	6.5
Dextrin	Trace	4.1	6.85	8.3
Glycogen	Trace	Trace	Trace	Trace
Inulin	....	....	Trace	Trace
Sucrose	....	....	Trace	Trace
Lactose	....	....	....	....
Maltose	....	....	Trace	Trace

Another series (table vi) of flasks was set up with the same form, using a "diffusion-extract" from the fresh tissue. Ten cc. of this diffusion represented 6 grams of the Laminaria pulp. In addition to the usual substrates, .5 per cent laminarin was used. Toluene was added and the flasks maintained for 60 days at room temperature (22–23°C.).

TABLE VI

THE ACTION OF A "DIFFUSION-EXTRACT" FROM FRESH LAMINARIA TISSUE UPON CERTAIN CARBOHYDRATES

Substrate 100 cc.	Sugar as glucose in 5 cc. mgms.				
	7 days	15 days	30 days	45 days	60 days
Starch	.....	Trace	2.7	4.2	5.35
Dextrin	Trace	Trace	3.6	5.15	7.40
Laminarin	Trace	2.4	3.9	5.4	5.65
Glycogen	Trace	Trace	Trace	Trace	Trace
Inulin	.....	.....	.....	Trace	Trace
Sucrose	.....	.....	Trace	Trace	Trace
Lactose	.....	.....	.....	.....	.....
Maltose	.....	.....	Trace	Trace	Trace

The diastases of fresh *Laminaria* seem slightly more active than those isolated from the dried tissue; however, no other carbohydrases were evident than those shown in the previous table.

THE CARBOHYDRASES OF ASCOPHYLLUM NODOSUM AND MESOGLOEA DIVARICATA

The Mesogloea material was dehydrated as soon as brought into the laboratory, the preliminary fresh-water washing being omitted because of the rapid gelatinization of the tissue. The crushed dried tissue, extracted in the usual way, gave a very heavy, stringy precipitate with alcohol, consisting, as did that from *Laminaria,* mostly of slime. This, when diffused in the usual volume of water, gave a very viscous mixture. Crushed fresh *Ascophyllum* was extracted directly. The viscosity of the extract was high, but the alcohol precipitate from it came down in a flocculent mass that gave only a slightly viscous diffusion with water.

Experiments were set up with the various carbohydrates heretofore employed, including laminarin, and in the different series, amounts of the "diffusion-extract" were used varying from 5–15 cc. As was true with the *Fucus* reported in the previous study, in no case were there evidences of hydrolysis even after 60 days at room temperature.

THE CARBOHYDRASES OF RHODYMENIA PALMATA

The air-dried Rhodymenia tissue proved to give rise to one of the most viscous extracts encountered in the algae, 20 volumes of water being necessary to make handling possible. With alcohol, a very rubbery, white precipitate came down that was made up of a large proportion of algal slime. This diffused very slowly, giving an extremely viscous mixture. Ten cc. of the "diffusion-extract" were used with the substrate to determine action, and toluene was added. The flasks were kept at a temperature of 21–22°C. for 100 days, sugar determinations being made from time to time, the results of which are given in table VII.

The results here are quite comparable to those obtained with *Ulva* and *Enteromorpha*, the same carbohydrates being

acted upon, although perhaps a little more slowly. This action is definitely progressive with starch, dextrin, and laminarin, but with glycogen it takes a sudden jump during the 15–45-day period, then remains practically stationary for the rest of the time the series is being maintained. As was true of the results shown in the previous tables, this carbohydrate was less favorable as a substrate than any of the other polysaccharides employed.

TABLE VII

THE ACTION OF A "DIFFUSION-EXTRACT" FROM AIR-DRIED RHODYMENIA TISSUE UPON VARIOUS CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.			
	15 days	45 days	75 days	100 days
Starch	9.2	12.2	14.8	18.2
Dextrin	8.25	9.7	10.3	11.1
Glycogen	Trace	6.1	6.4	6.7
Laminarin	4.7	7.3	9.6	10.5
Inulin	....	....	Trace	Trace
Sucrose	....	Trace	Trace	Trace
Lactose	....	....	....	Trace
Maltose	....	....	Trace	Trace

THE CARBOHYDRASES OF AGARDHIELLA TENERA

The very succulent nature of the freshly collected material compelled its partial dehydration immediately. Two 15-minute treatments with 95 per cent alcohol were used, then the tissue spread out on paper toweling to dry at room temperature. After drying, it was very easily powdered without the aid of quartz sand. The alcohol precipitate from a water extract of this powder was quite fine and flocculent, differing much from that of *Rhodymenia,* both in amount and in nature.

TABLE VIII

THE ACTION OF A "DIFFUSION-EXTRACT" FROM DEHYDRATED AGARDHIELLA TISSUE UPON CERTAIN CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.			
	15 days	45 days	75 days	100 days
Starch	6.35	9.4	14.5	20.9
Dextrin	7.00	10.5	15.95	19.7
Glycogen	2.35	6.15	6.65	6.85
Laminarin	5.8	8.3	11.6	13.2
Inulin	....	Trace	Trace	Trace
Sucrose	Trace	Trace	Trace	Trace
Lactose	....	....	....	Trace
Maltose	....	....	Trace	Trace

It diffused readily in water with no resulting viscosity. Ten cc. of the "diffusion-extract" were used for enzyme action, toluene added, and the flasks kept at a temperature of 21–23°C. for 100 days. The data here obtained are given in table VIII.

Dextrin here more nearly approaches starch as a favorable substrate, differing from the action evidenced by the other algae with the exception of *Laminaria,* where all action was slow. There is also a slightly increased action over that evidenced by *Rhodymenia,* for all the carbohydrates hydrolysed.

THE CARBOHYDRASES OF CERAMIUM RUBRUM

As was the case with *Rhodymenia,* it was necessary here to use 20 volumes of the water-extracting medium, not, however, because of the great viscosity, but on account of the great adsorption of water by the tissue particles. The alcohol precipitate was copious and finely flocculent. It diffused in water rather slowly, giving a mixture that was only slightly viscous. Ten cc. of the "diffusion-extract" were used for action, the usual percentage of toluene added, and the flasks maintained at a temperature of 21–23° C. for 100 days. The data are given in table IX.

TABLE IX

THE ACTION OF A "DIFFUSION-EXTRACT" FROM FRESH CERAMIUM TISSUE UPON CERTAIN CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.			
	15 days	45 days	75 days	100 days
Starch	6.85	8.1	11.75	16.9
Dextrin	11.5	15.0	17.5	19.6
Glycogen	Trace	6.2	7.3	8.85
Laminarin	7.2	9.4	12.1	12.2
Inulin	....	....	Trace	Trace
Sucrose	....	Trace	Trace	Trace
Lactose	....	....	....	Trace
Maltose	....	Trace	Trace	Trace

Dextrin proved the most favorable substrate for the carbohydrate enzymes of this alga, the hydrolysis being about the same as that evidenced by *Agardhiella.* With the exception of glycogen, the other carbohydrates showed a decreased hydrolysis when compared with this latter form, and when

compared with *Ulva* the difference is quite marked. As in the other algae, *Ceramium* showed no ability to hydrolyse the disaccharides used.

A COMPARISON OF THE DIASTATIC ACTIVITY OF ULVA LACTUCA WITH THAT OF LEAF TISSUE FROM SOLANUM TUBEROSUM

One of the very evident facts brought out by the data in the preceding tables was the relative slowness with which hydrolysis was carried on. This point made it seem worth while to compare, in a general way, the activity of such a form as *Ulva* with the starch-forming leaf tissue of a higher plant, one from which diastase could be isolated rather easily. The potato (*Solanum tuberosum*) was chosen.

The Ulva tissue was from an air-dried lot that had been tried out earlier and had been found quite active. Fresh potato tops were brought into the laboratory, and both these and the *Ulva* given the "dauerhefe" treatment. After dehydrating and drying at room temperature, both lots were ground in a mill, then reduced to a fine powder in a mortar. Exactly 18.5 grams of each were extracted with 250 cc. of water for 12 hours at room temperature with toluene added as an antiseptic, and then the protein-enzyme complex precipitated with 2.5 volumes of 95 per cent alcohol. The Ulva precipitate was the characteristic heavy white mass to which attention has been called before, while that of the potato was finely divided and dark.

The entire amount of each precipitate was diffused in 60 cc. of water. The Ulva precipitate gave a rather viscous diffusion, due to the adsorption of water by the protein particles; that from the potato did not all go into solution, making it necessary to shake the flask so that a true sample might be obtained. Five cc. of the "diffusion-extract" represented 1.84 grams of the original dehydrated tissue, and this volume was used with 50 cc. of a starch and dextrin substrate. Toluene was added as an antiseptic, and the flasks kept at a temperature of 31°C. for 42 days. Portions of the substrate were removed from time to time and sugar determinations made, the results of which are shown in table x.

The action of the potato extract upon starch was about two and one-half times that of *Ulva,* and its action on dextrin about twice in all of the determinations made. For some unknown reason the hydrolysis of dextrin by the diastase from *Ulva* ceased after the twenty-eighth day.

TABLE X

A COMPARISON OF THE DIASTATIC ACTIVITY OF ULVA WITH THAT OF POTATO LEAF TISSUE

Substrate 50 cc.	Sugar as glucose in 5 cc. mgms.									
	14 days		21 days		28 days		35 days		42 days	
	Ulva	Potato	Ulva	Potato	Ulva	Potato	Ulva	Potato	Ulva	Potato
Starch....	8.7	18.1	9.6	26.3	11.8	28.1	12.9	33.5	13.8	35.5
Dextrin...	10.5	17.3	11.5	25.1	17 8	27.5	17.9	30.3	17.9	31.9

ACTION OF VARIOUS ALGAL EXTRACTS UPON THE CARBOHYDRATE CONSTITUENTS OF AGAR-AGAR, AND OF VARIOUS GUMS, AS WELL AS EXPERIMENTS UPON THE AUTOLYSIS OF ALGAL SLIME

Because of the large amounts of carbohydrate-containing slime formed by many algae, and because of the rôle this might play as a reserve product, it was deemed advisable to try out the various algae for enzymes capable of hydrolysing such complex carbohydrates to assimilable sugars. It was assumed on the basis of the work done by König and Bettels ('05) and others, that such hydrolytic products would be reducing sugars, in all probability galactoses and pentoses.

A series was set up with each of the several algae, using 50 cc. of .25 per cent agar as a substrate and varying amounts of a "diffusion-extract" from fresh tissue. The agar substrate was slightly viscous in the cold, but when kept at a temperature of 40° C., the optimum temperature for diastase, this was not noticeable. Toluene was used as an antiseptic. The flasks were shaken at regular intervals during a 30-day period and at the end of that time aliquot portions were removed and tested for reducing sugars. There was no reduction in any case.

As a parallel series, thin strips of agar were placed in test-tubes and 20 cc. of "diffusion-extract" added. Toluene was used as an antiseptic and the tubes kept at a temperature of 40° C. for two months. At the end of that time no hydrolytic

action was observable, either by reduction of Fehling's or by microscopical examination.

In the experiments on the hydrolysis of various poly- and disaccharides, checks were set up in which the usual amount of "diffusion-extract" was placed in distilled water. This was to determine the reduction of copper, if any, due to the "diffusion-extract" itself. In no case was there more than a very slight trace that might have been due to other causes than enzymic. However, it was thought that a self-digestion series would more definitely determine whether the hydrolysis of the carbohydrates of the slime could be brought about by specific algal enzymes. With this in mind, a series was arranged in which the flasks contained 50 cc. of a water extract from each of the forms investigated. Checks were set up in which the "diffusion-extract" was inactivated in the autoclave. Toluene was used as an antiseptic and the flasks maintained at a temperature of 22–23°C. for two months. Aliquot portions removed from time to time failed to show the slightest trace of hydrolysis.

It will be remembered that Tihomirov ('10) had found osozone-forming sugars in the conceptacles of *Ascophyllum* and *Fucus* that he thought might be dextrose and d-galactose, possibly also fucose and arabinose. Thinking that these might possibly have arisen from their corresponding anhydrides contained in the conceptacle slime, a self-digestion series was set up with an extract from the abscised, crushed conceptacles of those two forms. The *Fucus* was in a fruiting state. The series were set up in duplicate, one kept at room temperature and the other at 32–33°C. Fehling's test showed no hydrolysis after a month.

Pentosans alone were then used as substrates. Two series of flasks for each of the algae investigated were set up, each containing a .5 per cent solution of gum arabic.[1] To one series was added 10 cc., to the other 20 cc. of "diffusion-extract," and the flasks placed at room temperature with toluene as an antiseptic. No hydrolysis was apparent either

[1] The gum arabic was dissolved in water, then precipitated with several volumes of 95 per cent alcohol to get rid of reducing sugars.

by the phloroglucin test or by sugar determinations, even after 60 days.

THE ACTION OF ALGAL "DIFFUSION-EXTRACTS" UPON CELLULOSE AND HEMICELLULOSE

Experiments were carried out to determine the presence or absence of cellulose hydrolysing enzymes in the algae, and to this end several methods were employed. First, strips of filter paper were placed in test-tubes and entirely covered with 20 cc. of "diffusion-extract." Checks were maintained with distilled water and also with the "diffusion-extract" alone. The series were set up in duplicate—one kept at room temperature and the other at 35° C., both with toluene as an antiseptic. After definite intervals during a 60-day period, the contents of the tubes were tested for reduction. None was observable in any case, and microscopic examination of the filter paper failed to reveal any decomposition whatsoever.

A double series was then set up in a similar way, except that 2 grams of fresh, crushed algal tissue were added to the tubes instead of the "diffusion-extract," together with 20 cc. of distilled water. At the periods noted above, microscopic examination revealed no attack. It was thought an inherent difference between algal and filter paper cellulose might be responsible for this absence of action. Accordingly, cellulose was prepared from the tissue of *Ascophyllum* after the method described by Fowler ('11, p. 159) and used by Cooley ('14). Fifty grams of air-dried tissue were placed in a liter flask, 500 cc. of distilled water added, and the lot placed in the autoclave at 15 pounds for 15 minutes to destroy any cellulase that might be present, and also to extract as much as possible of the water-soluble substances. The water was filtered from the tissue, fresh water added, and the flask placed in an incubator at 35° C. It was kept at this temperature with daily changes of water for 10 days, at which time the water-soluble constituents seemed to be almost entirely removed. The treatment from here on was the same as that described by Cooley. To the tissue was added a liter of potassium-chlorate-nitric-acid solution made up in the proportion of 30 grams of potassium chlorate to 520 cc. of nitric

acid (sp. gr. 1.1). The flask was kept in the ice-box for two weeks, when the oxidizing mixture was changed and the new lot allowed to remain another fortnight. At the end of this time a yellowish white tissue was obtained, representing fairly pure algal cellulose. This was filtered off, washed well with distilled water, and dried in the oven at 75–80°C. The final product weighed 19.7 grams.

This cellulose was used in a way similar to the filter paper in the first series. One gram was placed in each flask and well shaken up with 50 cc. of distilled water. A concentrated "diffusion-extract" was prepared from *Ascophyllum, Laminaria, Ulva,* and *Chondrus,* 10 cc. of which represented 5 grams of the original dried tissue. This volume was added to the flasks, and the series set away at 30°C. with toluene as an antiseptic. At the end of two months no reduction of Fehling's was observable and under the microscope there seemed to be no decomposition of the cellulose particles.

Action on hemicelluloses.—Hemicellulose was used from two sources—from date seeds, and from the seeds of the wild persimmon, *Diospyros virginiana.* In both cases the experiments were essentially the same. The horny coats were broken and the embryos removed. Small pieces of the hemicellulose were then taken, placed in a flask with water, and heated in the autoclave at 15 pounds for 15 minutes to kill the cytase present. Upon removal from the autoclave the pieces were washed several times in distilled water, being left in the last wash water for several days with toluene as an antiseptic—this to get rid of any reducing sugars present. Two of these washed pieces were placed in test-tubes with 10 cc. of the concentrated "diffusion-extract" used in the experiments with cellulose. Another lot was covered with 10 cc. of distilled water and 2 grams of the dried algal powder added. In a third series shavings of the hemicelluloses were mounted in a Van Tieghem cell with a drop of enzyme solution. All the algae under investigation were tried out, but in no case was there the slightest trace of decomposition, either microscopically or by the reduction of copper.

Results.—These negative results do not necessarily argue against the production of slime through the agency of enzymes. It is impossible to exactly reproduce the conditions of the cell *in vitro,* and enzymes which might act upon cellulose in the living tissue to produce slime might easily be inhibited from action on cellulose or hemicellulose under the conditions of the experiments. Grüss ('10) found that fresh cherry gum contained cytase, but that none was demonstrable in the older gum. He also found that malt diastase would not act upon such gum until the tannins had been removed. It is known that the algae do contain tannins or "tannoidal" bodies, the writer having demonstrated a "tannoid" content in *Ascophyllum* of 1.1 per cent of the dry weight. These, or other agents, could be involved in the partial or complete inhibition of cytolytic action. On the other hand, indirect evidence, at least, points to the presence of the galactan and pentosan groups as due to their being laid down as such, that is, they do not arise as the direct result of hydrolytic enzyme action, but probably represent the final step in the condensation of those particular hemicelluloses. Tschirsch ('89) and his students have shown that the algal slime exists as an intracellular substance, and they hold that in most instances, at least, it does not arise from the cellulose. This seems to be the logical view, and we in turn seem justified in looking upon the galactan and pentosan groups in the algae as normal products of the plant's metabolism, present at all stages in the plant's growth, and capable of giving rise to gelatinization at any time upon the adsorption of water. If one examines, for instance, such forms as *Fucus, Mesogloea,* and *Chondrus,* the slime is hardly detectable when the plants are growing under normal conditions, but when brought into the laboratory and placed in fresh water, a rapid adsorption begins at once. The dissolved salts in sea-water are undoubtedly the inhibiting factors in such adsorption under normal conditions.

That this inhibition is not bound up with the living cell may be shown by the simple experiment of killing two fronds of *Chondrus,* for example, and placing one in fresh, the other

in salt water, with toluene to keep down bacterial action. Very slight, if indeed any, gelatinization is evident with the frond placed in salt water, while that in fresh water begins to gelatinize immediately. It is also a well-known fact that in histological or cytological work with these forms, the killing fluids must be made up in sea-water or water containing a high percentage of salts, else gelatinization interferes. These facts, together with the apparent absence of cellulase and cytase, tend to show that the galactan and pentosan groups are always present as final condensation forms of their particular "generic" carbohydrate line, and that sliming in the marine algae, at least, is the result of the adsorption of water by these already existing carbohydrate groups.

DISCUSSION OF RESULTS OF CARBOHYDRASE EXPERIMENTS

It is seen from the data presented in the foregoing tables that carbohydrases in the algae, at least those that can be isolated by standard methods, are very few. Furthermore, in all cases where such carbohydrase action is evident, it is limited to the polysaccharides—starch, dextrin, laminarin, and glycogen. In no case were the disaccharides hydrolysed. As groups, the "greens" are more active than the "reds," while of the "browns," *Laminaria* is the only form in which carbohydrate action is demonstrable. Moreover, the action here is extremely slow and is limited to starch, dextrin, and laminarin. *Mesogloea* and *Ascophyllum* are similar to *Fucus* in failing to show the presence of carbohydrases. Within the groups there is little difference in the rate of carbohydrase action. This is especially true in the "greens." Of the "reds," *Agardhiella* is a little more active than the other forms investigated, while *Ceramium* is slightly the slowest. Bartholemew ('14), in the work already referred to, also found that *Ceramium* was less active than the other "reds" with which he worked.

The various polysaccharides, with two exceptions, prove favorable as substrates for the various algae in the same order, viz., starch, dextrin, laminarin, and glycogen. The carbohydrases of *Ceramium* act more rapidly upon dextrin than upon starch and this is also true of *Laminaria,* although

to a lesser extent. Glycogen, which is very generally hydrolysed by diastase, is here decidedly less readily attacked than the other polysaccharides. This would seem to indicate that we are dealing with a distinct enzyme, one that might be placed in the same category with dextrinases. These latter always occur with the diastases but are held by many workers to be distinct.

Some of the substrates tested for hydrolysis do not, as far as we know, occur in the plants investigated. This is true of sucrose, lactose, and inulin. However, although this might reconcile us to the failure to find their specific enzymes, it does not argue conclusively against such enzymes being formed. It is well known that tissues do form ferments that have no detectable substrates upon which to act—the rennen of the bird's stomach and the urease of the Soja bean being notable examples. Inulin, as pointed out previously, does occur in certain "greens," as in *Acetabularia* and members of the *Dasycladaceae*. Unfortunately, none of these forms were available for investigation.

The absence of lactase and sucrase is not so significant as is that of maltase. It is very generally considered that in the plant, as well as in the animal organism, poly- and disaccharides must be hydrolysed to simple sugars before assimilation can take place. It is hardly possible that the algae are an exception to this general rule and yet it is difficult to account for this important negative result. It is known that inhibiting agents do not affect all enzymes alike, and it may be here that if such agents are liberated on the death of the cell, the maltase might prove more sensitive to them than the other carbohydrate enzymes. According to the findings of Kylin ('13), both dextrose and fructose have been demonstrated in the tissues of *Ascophyllum*, *Fucus*, and *Laminaria*, but in extremely small quantities. These results would tend to convince one that an enzyme giving rise to them is probably present in the algal cell.

Such carbohydrates as galactans, pentosans, and mannans, are very frequently met with in the algae and are potentially capable of being split to assimilable sugars. That they are

not so split, however, seems evident, at least not through the activity of demonstrable algal enzymes, and in the face of the negative evidence obtained, we would consider them as by-products of metabolism rather than as playing the rôle of reserves. As such, they would not be so comparable to the reserve carbohydrates of the date as they would be perhaps to the mucilaginous constituents of various seeds, as those of flax, mistletoe, etc. These latter adsorb water readily with gelatinization, and as far as is known, never function as reserves but act in a purely mechanical way (Czapek, '13, p. 705).

LIPASES IN THE ALGAE

The almost universal presence of fats in the marine algae led to the question of their assimilation. Accordingly, experiments were set up to determine the lipolytic activity upon emulsions of neutral fats as well as upon certain esters of the lower fatty acids. For the neutral fats olive oil was chosen as a substrate, and two general methods were employed in forming the emulsion.

The first, an olive oil-casein emulsion was made up after a method described by Bloor ('14). Four grams of casein were placed in a warm mortar on a water bath and water added until the whole formed a paste of medium viscosity. A drop of phenylphthalein was added, then N/1 NaOH poured in and stirred with the casein until the latter had been dissolved, this point being indicated by a permanent pink tinge of the mixture. Eight cc. of olive oil were stirred into the hot solution and then ground with a pestle until all the oil globules had disappeared. At this point the mortar was removed from the bath and the emulsion cooled. During the cooling it was found necessary to stir the mixture occasionally. The thick, creamy mass resulting was diluted up to the required concentration by the careful addition of water. If this dilution is too great, the oil globules tend to rise to the surface.

The second method was also suggested by Doctor Bloor, but, as far as is known, has not been described. Eight cc. of olive oil were dissolved in the smallest amount of absolute alcohol necessary. This solution was run through a hot fun-

nel to which a drawn-out piece of glass tubing had been attached, into about 100 cc. of cold distilled water, the water being stirred constantly while the olive oil was being run in. A milk-white emulsion made up of extremely small suspended globules of oil resulted. In an emulsion carefully made, most of these globules are small enough to show Brownian movement. The alcohol was driven off finally by heating and the emulsion made up to the desired concentration.

Both emulsions stand up well. In the latter, however, there is a tendency toward flocking out by some of the smaller particles upon the addition of any salt-containing substance, such as, for instance, algal powder; but, on the other hand, it has the advantage of being more easily checked up because of its simpler composition.

TABLE XI

LIPOLYTIC ACTION OF THE SEVERAL ALGAE UPON OLIVE OIL-CASEIN EMULSION

Alga	Number cc. of N/10 NaOH to neutralize 10 cc. of substrate											
	4 days				10 days				15 days			
	Emulsion + tissue	Emulsion	Water + tissue	Net acidity	Emulsion + tissue	Emulsion	Water + tissue	Net acidity	Emulsion + tissue	Emulsion	Water + tissue	Net acidity
Ulva	....	....	....	....	1.3	.25	.1	.95	....	....	....	....
Enteromorpha	....	....	....	....	1.25	.2	.1	.95	....	....	....	....
Mesogloea	.6	.00	.1	.5	1.00	.1	.1	.8	2.2	.6	.075	1.525
Ascophyllum	....	....	....	....	1.50	.05	1.0	.45	....	....	....	....
Laminaria	....	....	....	....	.4	.00	.05	.35	.6	.1	.025	.475
Chondrus	1.2	.00	.05	1.15	1.85	.00	.05	1.8	2.3	....	.1	2.200
Agardhiella	....	....	....	....	.3	.00	.05	.25	....	....	....	....
Ceramium	....	....	....	....	1.6	.00	.1	1.5	....	....	....	....
Rhodymenia	.3	.1	.05	.15	.9	.2	.15	.55	....	....	....	....
Champia	.2	.1	.05	.05	.35	.3	.05	.00	....	....	....	....

In all the lipolytic experiments, algal powder or fresh algal tissue crushed with fine quartz sand, was used as a source of enzyme action. In some of the original series the olive oil-casein emulsion was employed, but on account of the danger arising from a possible hydrolysis of the casein with a resulting increase in acidity, the alcohol emulsion was used in the later work.

Lipolytic action of the several algae upon olive oil-casein emulsion.—In this experimental series (table XI) flasks were set up containing 50 cc. of olive oil-casein emulsion as a sub-

strate, 5 grams of crushed algal tissue for enzyme action, and 10 cc. of 95 per cent alcohol as an antiseptic. Checks were employed wherein the flasks in one case contained the emulsion alone, and in another case, the same weight of algal pulp in distilled water. The flasks were maintained for 15 days except for the forms especially noted. At intervals 10 cc. portions were removed and titrated against N/10 NaOH with phenylphthalein as an indicator.

Lipolytic action on alcohol-water-olive oil emulsion.—Because of the possibility of the hydrolysis of the casein in the emulsion used in the preceding experiments, a series (table XII) employing the alcohol-water emulsion was set up as a check. This emulsion alone was practically neutral but a

TABLE XII
LIPOLYTIC ACTION OF THE SEVERAL ALGAE UPON ALCOHOL-WATER-OLIVE-OIL EMULSION

Alga	Number cc. N/10 NaOH to neutralize 10 cc. substrate after 10 days				
	Emulsion + tissue	Emulsion alone	Water + boiled tissue	Water + tissue	Net acidity
Ulva	.9	.00	.05	.05	.85
Enteromorpha	.8	.00	.025	.1	.7
Mesogloea	.65	.00	.05	.075	.575
Ascophyllum	.2	.00	.15	.15	.05
Laminaria	1.15	.00	.02	.3	.85
Chondrus	1.45	.00	.1	.35	1.10
Agardhiella	.25	.00	.05	.05	.20
Ceramium	.85	.00	.1	.20	.65
Rhodymenia	.525	.00	.05	.15	.375
Champia	.125	.00	.15	.15	.00

slight acidity was produced by the addition of the algal powder. A negligible amount of the oil globules ran together and collected at the surface of the liquid after some days, but the bulk of the emulsion stood up well. As in the preceding series, 5 grams of the fresh tissue were used as a source of lipolytic activity, and the alcohol in which the olive oil had been dissolved served as an antiseptic. Fifty cc. of the emulsion were used as a substrate, and the flasks maintained at a temperature of 22–23°C. for 10 days.

Lipolytic action on triacetin.—The lipolytic activity of dry tissue powder of *Ulva, Mesogloea,* and *Chondrus* was tested, using a .5 per cent solution of triacetin as a substrate. Two

grams of the tissue powder were used, otherwise the series (table XIII) was arranged exactly as the preceding and kept at room temperature for 25 days.

Action on other esters.—A series was set up with methyl acetate, ethyl acetate, and ethyl butyrate in .25 per cent solution, using 2 grams of algal powder with 50 cc. of the sub-

TABLE XIII

THE ACTION OF POWDERED TISSUE FROM CERTAIN ALGAE UPON TRIACETIN

Alga	Number cc. of N/10 NaOH to neutralize 10 cc. substrate after							
	10 days				25 days			
	Triacetin + tissue	Water + tissue	Triacetin alone	Net acidity	Triacetin + tissue	Water + tissue	Triacetin alone	Net acidity
Ulva	.3	.025	.1	.175	.5	.15	.15	.2
Mesogloea	.25	.05	.1	.1	.4	.2	.15	.05
Chondrus	.55	.35	.1	.1	.8	.4	.15	.25

strate in 20 per cent alcohol. Titrations were made from time to time against N/10 NaOH with phenylphthalein as an indicator. Even after 60 days at room temperature no increase in acidity was observable over the checks.

General results for experiments with lipases.—The results serve to show that, although slight, there is distinct lipolytic activity in most of the forms investigated. The various groups of algae are not so distinct regarding this activity as was the case with the carbohydrases, nor does the activity of the individual alga in this case relate itself particularly to the activity shown by the form in its carbohydrase action. *Agardhiella* hydrolyses the polysaccharides more rapidly than any other alga, yet its lipolytic activity is very low. Likewise, *Laminaria*, so inactive in the previous group of enzymes, is among the most active on fats. *Fucus*, on the other hand, was found in previous work to have no action on either carbohydrates or fats.

The action is especially evidenced by use of the olive oil-casein emulsion. In general, the increases were less where the alcohol-water emulsion was used—a difference probably ex-

plainable on the ground that the casein gave rise to a slight acidity.

Specificity of action might explain the failure to obtain action on most of the esters. Euler ('12) differentiates the lipases into true lipases and esterases, the former acting on neutral fats particularly, the latter on the methyl and ethyl esters of the lower fatty acids. Even in this latter restricted field, great specificity may be shown. Reed ('12) found that ethyl acetate was quite rapidly acted upon by an esterase isolated from *Glomerella rufomaculans,* while ethyl butyrate was only slightly hydrolysed.

THE PROTEINASES

The proteolytic activity of the various algae was tested on albumin, casein, legumin, peptone, gelatin, and in certain cases, on proteins isolated from the algal tissue—most of these under acid, alkaline, and neutral conditions. The first four were made up in 1 per cent concentrations. Albumin and peptone went into solution quite readily; legumin and casein, being insoluble in water, were either weighed out directly, or dissolved in N/10 NaOH. The albumin and gelatin were also tested in the form of Mett's tubes, and the gelatin alone in test-tubes where it was held at a temperature high enough to keep it in a liquid state while in contact with the algal powder. In all cases, algal tissue was used directly, either fresh crushed, or dry powdered—usually 2 grams of the powder or 5 grams of the fresh tissue to each 50 cc. of substrate.

Determination of hydrolysis.—Proteolytic action was determined in several ways, each acting as a check on the others. The biuret test was used for the demonstration of tryptic action, the proteins being precipitated by $(NH_4)_2SO_4$ in saturated solution and the test applied in the usual way. The tryptophane test was employed for ereptic action and this also furnished a check on the action of trypsin. In this, 1 cc. of the protein solution was placed in a small evaporating dish, a drop of glacial acetic acid added, and then a few drops of strong chlorine water. The hydrolysis to the amino acid stage

was also demonstrated in two other ways—by the formaldehyde-titration method of Sörenson ('08), and the determination of the amino-nitrogen by the micro-Kjeldahl method of Folin ('13). The Sörenson method consisted in adding 2 cc. of formalin, made alkaline to a faint pink tinge with N/20 NaOH, to 10 cc. of the filtered protein solution, made alkaline to the same color. Upon mixing, the color disappeared and the acidity resulting was titrated against N/50 NaOH, using phenylphthalein as an indicator.

In the determination of the amino-nitrogen by the "micro" method of Folin, the protein in a 5 cc. filtered portion of the solution was precipitated with 2 cc. of a 25 per cent solution of phosphotungstic acid in 5 per cent H_2SO_4. The precipitate was filtered off and a 2 cc. portion of the filtrate removed for the determination of the nitrogen. Duplicate determinations were made in all cases. These portions were placed in Jena test-tubes, 20×200 mm., 1 cc. concentrated H_2SO_4 added, then 1 gram of K_2SO_4, and a drop of 5 per cent $CuSO_4$. The digestion was carried on over the flame from a micro burner, the fumes being carried away by the fume adsorbers described by Folin. Usually 20 minutes sufficed for the completion of the digestion, although in a few instances 25 minutes were required. After cooling slightly, 6 cc. of distilled water were carefully added. The tubes were then transferred to the distilling apparatus where concentrated NaOH was added to alkalinity, and the tube contents distilled over for three minutes, the NH_3 being collected in a known volume of N/10 HCl. The acid in the collection flask was titrated against N/10 NaOH with alizarin red (alizarin sulfonsäure Natrium, Merck), and the amount of nitrogen represented by the acid neutralized, determined.

In the method originally described by Folin, the NH_3 was not distilled but was forced over from an alkaline solution by a strong air current. However, students in his laboratory have made use of a micro distilling apparatus, and the suggestion for the ones employed here owes its origin to one of Folin's assistants. Distillation has the advantage of quickness, and from the writer's experience, of accuracy as well,

at least where suction instead of compressed air is employed in the air method. The results with the air current were very often below the theoretical. The distilling tubes used were made in the laboratory from glass tubing, the outer jacket measuring 40×2 cm., and the inner being 5 mm. in diameter. The lower end of this latter, where it dipped into the collection acid, was fitted with a larger tube 14 mm. in diameter—this to prevent a back flow of the acid; to the upper end of this inner tube was attached a safety bulb made from a 10 cc. pipette, and this in turn fitted into the Jena tube containing the distilling mixture, by means of a two-hole rubber stopper. Through the second hole in this stopper was a small piece of glass tubing closed at the upper end with a bit of rubber tubing and a pinch clamp; it was through this that the alkali was added after the apparatus was connected up for distillation.

Considerable trouble was experienced at first with bumping, especially after the digestion mixture had become concentrated. Neither bits of glass nor pebbles would overcome it. Finally the expedient was adopted of using short pieces of glass tubing sealed at one end and this end placed uppermost. These were of such a diameter that after digestion, the digestion mixture drawn up into them by the cooling of the contained air, would easily drain out when the boiling tube was forced up on the side of the test-tube by a quick downward motion.

The action of Enteromorpha, Mesogloea, and Chondrus powder upon various proteins.—Fifty cc. lots of casein, legumin, albumin, and peptone were used as substrates in this series—all in 1 per cent concentrations. The albumin and peptone were dissolved directly in distilled water, the legumin and casein in N/10 NaOH. Two grams of air-dried tissue powder were used for proteolytic action, with the exception, however, of *Mesogloea,* which, as before stated, was partially dehydrated before being air-dried. The various substrates were made neutral by the addition of N/10 alkali and then acid or alkaline by further addition of 2.5 cc. of N/10 HCl or NaOH. In the formaldehyde titrations 10 cc. of the sub-

TABLE XIV

THE ACTION OF TISSUE POWDER FROM CERTAIN ALGAE UPON VARIOUS PROTEINS

Substrate 50 cc.	Weight algal powder gms.	Reaction* of substrate	*Enteromorpha*				*Mesogloea*				*Chondrus*			
			Formol titration†		Biuret	Tryptophane	Formol titrations		Biuret	Tryptophane	Formol titrations		Biuret	Tryptophane
			15 days	30 days			15 days	30 days			15 days	30 days		
Albumin	2	neut.	2.20	3.70	+	+	1.40	2.30	+	+	2.70	5.10	+	+
	.....	neut.	.30	.40	—	—	.50	.70	—	—	.40	.65	—	—
	2	acid	.80	1.90	+	—	.70	.90	—	—	1.65	2.85	—	—
	.....	acid	.40	.55	—	—	.40	.40	—	—	.65	.80	—	—
	2	alk.	2.75	4.95	+	+	2.15	4.80	+	+	2.10	4.35	+	+
	.....	alk.	.25	.30	—	—	.45	.55	—	—	.75	.75	—	—
Peptone	2	neut.	3.25	7.10	.....	+	2.70	5.75	.....	+	4.95	12.05	.....	+
	.....	neut.	.90	1.10	.....	—	.75	.80	.....	—	.90	1.05	.....	—
	2	acid	1.35	4.75	.....	+	1.50	4.35	.....	+	1.75	3.80	.....	+
	.....	acid	1.15	2.05	.....	—	.80	.95	.....	—	.60	.60	.....	—
	2	alk.	4.50	9.30	.....	+	3.55	7.90	.....	+	5.30	12.55	.....	+
	.....	alk.	.95	1.20	.....	—	1.10	1.10	.....	—	.85	1.20	.....	—
Casein	2	neut.	3.175	11.80	+	+	2.30	2.45	—	—	3.10	7.15	+	+
	.....	neut.	2.40	2.70	—	—	2.50	2.55	—	—	2.20	2.25	—	—
	2	acid	2.75	3.40	—	—	2.10	2.35	—	—	2.75	2.65	—	—
	.....	acid	2.60	2.70	—	—	2.40	2.45	—	—	2.80	2.95	—	—
	2	alk.	4.65	12.30	+	+	2.60	2.75	—	—	5.30	10.15	+	+
	.....	alk.	2.10	2.45	—	—	2.30	2.20	—	—	2.10	2.35	—	—
Legumin	2	neut.	2.10	4.95	+	+	1.55	1.70	—	—	2.10	2.45	—	—
	.....	neut.	1.80	1.95	—	—	1.90	2.00	—	—	1.85	1.65	—	—
	2	acid	2.00	3.60	+	+	1.40	1.65	—	—	1.70	2.30	—	—
	.....	acid	1.60	1.65	—	—	1.65	1.75	—	—	1.55	1.60	—	—
	2	alk.	3.35	7.10	+	+	1.95	2.15	—	—	2.90	5.85	+	+
	.....	alk.	2.10	2.05	—	—	1.50	1.90	—	—	.40	.80	—	—
Water	2	.....	.20	.25	—	—	.35	.30	—	—	.65	.75	—	—

*The substrates were made N/200 acid or alkaline with N/10 HCl or NaOH.

†The values represent the cc. of N/50 NaOH to neutralize 10 cc. of the substrate used.

strate were titrated against N/50 NaOH. One per cent chloroform-thymol was used as an antiseptic, and the flasks were kept at a temperature of 22–23°C. for 30 days.

The forms used in table XIV show a general ability to hydrolyse proteins. All four proteins employed were acted upon by one alga or another, but peptone and casein in neutral and alkaline solution were the most readily attacked. *Enteromorpha* split albumin and legumin but poorly; *Chondrus* acted upon legumin only in alkaline solution, and then slightly; *Mesogloea* failed to hydrolyse casein and legumin, and its action on albumin and peptone was very slow.

The action of Ulva, Laminaria, and Agardhiella powder on peptone and casein in alkaline and neutral solution.—Indications in the preceding experiment seemed to point to the fact that peptone and casein were more easily acted upon than the other proteins—and these more especially in neutral and alkaline solution. Accordingly, a series was set up with these two substrates, similar in all respects to the preceding one, except that the acid substrate was omitted and that *Ulva, Laminaria,* and *Agardhiella* were used for proteolytic action. Five grams of air-dried tissue were employed with 100 cc. of substrate. One per cent chloroform-thymol served as an antiseptic, and the flasks were kept at 35°C. for 30 days. Formaldehyde titrations were made after 15 and 30 days and tryptophane tests and amino-nitrogen determinations after 30 days. In the titrations 10 cc. of substrate were titrated against N/50 NaOH, and the amino-nitrogen represents that in 2 cc. of the filtrate from phosphotungstic precipitated protein.

The data in table XV tend to substantiate that of table XIV concerning the hydrolysis of peptone and casein. The higher temperature at which the flasks were maintained undoubtedly had something to do with the larger amounts of amino acids split off from these two proteins than was the case in the preceding series, yet if we can judge by the action on carbohydrates and fats, we are dealing here with the more active members, enzymatically, of their respective groups.

On the whole, peptone and casein seem to be the most favorable substrates of those used for proteolytic activity, and

TABLE XV

THE ACTION OF POWDERED TISSUE FROM CERTAIN ALGAE UPON PEPTONE AND CASEIN

Substrates 100 cc. 1 per cent	Weight algal powder gms.	Reaction of substrate	*Agardhiella*				*Ulva*				*Laminaria*			
			Formol* titrations		Tryptophane test	Amino-N† in 2 cc. mgms.	Formol titrations		Tryptophane test	Amino-N in 2 cc. mgms.	Formol titrations		Tryptophane test	Amino-N in 2 cc. mgms.
			15 days	30 days			15 days	30 days			15 days	30 days		
Peptone	5	neut.	4.35	15.00	+	1.68	2.80	6.75	+	1.06	2.25	5.65	+	1.14
	.....	neut.	.70	1.00	+	.07	.70	1.00	+	.07	.70	1.00	+	.07
	5	alk.	5.30	18.00	+	1.84	3.05	8.35	+	1.19	2.30	4.15	+	.39
	.....	alk.	.55	.85	—	.00	.55	.85	—	.00	.55	.85	—	.00
Casein	5	neut.	8.70	22.00	+	1.49	6.40	17.70	+	.92	5.95	15.60	+	.98
	.....	neut.	5.55	6.10	—	.48	5.55	6.10	—	.49	5.55	6.10	—	.49
	5	alk.	8.60	23.15	+	1.56	6.25	26.35	+	1.10	5.70	14.50	+	.83
	.....	alk.	5.20	5.70	—	.35	5.20	5.70	—	.35	5.20	5.70	—	.35
Water	5	.....	.40	.95	—	.08	.90	1.65	—	.11	1.05	1.40	—	.09

* Values represent the number of cc. N/50 NaOH to neutralize 10 cc. of substrate.

† The amino-nitrogen in 2 cc. of filtrate from phosphotungstic precipitated protein represents 1.4 cc. of the original substrate.

these in neutral and slightly alkaline solution. This was shown in the formol titrations[1] and in the determination of the nitrogen in the amino acids split off. Albumin was slowly acted upon by *Enteromorpha, Mesogloea,* and *Chondrus.* The first and last also hydrolysed the vegetable protein, legumin, to a slight extent—an action that was not shared by *Mesogloea.*

The action of algal powder on the proteolysis of gelatin and albumin in Mett's tubes.—Two lots of Mett's tubes were made up, one containing coagulated egg-white, and the other, 15 per cent gelatin. In each of a series of flasks containing 50 cc. of distilled water, N/200 NaOH and N/200 HCl respectively, were placed one tube each of egg-white and gelatin. Two grams of the powdered tissue from each of the several forms under investigation were added for enzyme action and the usual percentage of toluene used as an antiseptic. The several series were kept for two months at room temperature. At the end of that time the albumin tubes in the alkaline solution containing the algal powder of *Ulva, Enteromorpha, Chondrus,* and *Agardhiella* showed a slight digestion. The checks in the alkaline solution alone showed swelling. However, although this was indicative of action, it was not definite, since the great length of time the protein was in contact with the complex constituents of the tissue may have been a factor in either causing a slight hydrolysis or a contraction of the albumin. On the other hand, *Laminaria, Ascophyllum, Mesogloea,* and *Ceramium* caused no such action. The gelatin tubes showed no evidences of action even after 60 days.

The effect of proteinases on the hardening of gelatin.—Dox ('10) describes a method for testing the hydrolysis of gelatin which consists in keeping the protein in a liquid state during contact with the material being tested for proteolytic activity, then at the end of a stated period noting whether the gelatin congeals when placed in cold water. This method was used in the following way: Five cc. of 20 per cent gelatin were placed in each of a series of test-tubes, and 5 cc. of the standard

[1] The formaldehyde titrations, as used here, were satisfactory only in a general way, i. e., to show relative rather than exact differences in the amounts of amino acids split off. The differences brought out by the amino-nitrogen determinations are much more exact.

"diffusion-extract," described under "carbohydrases," used for action. The contents of the tubes were made neutral, and acid and alkaline to N/200, as was done in the other proteolytic experiments. Five drops of chloroform-thymol were added as an antiseptic. Checks were set up containing the gelatin together with 5 cc. of boiled "diffusion-extract." The tubes were placed in an incubator at 35° C. for a week, at which time they were removed and cooled in running water. All tubes hardened in a short time, showing that no hydrolysis had taken place.

General results for experiments on proteolysis.—The proteolytic activity, although slow, as was the case with the other enzymes investigated, is definite enough to warrant the statement that proteinases and peptases are very generally present in the algae. When present, such enzymes act best under neutral and alkaline conditions. This last finding is interesting in the light of the existing differences of opinion regarding the relative value of acid and alkaline substrates for vegetable proteinases. It will be recalled that Vines ('97) found that acidity favored the proteinase contained in the leaf pitchers of *Nepenthes*, and in a later paper, he states that peptase (hydrolysing albumoses and peptones to amino acids) always act best under faintly acid conditions. Emmerling ('02), on the other hand, demonstrated that the papain of *Carica papaya* acted more rapidly when the substrate was alkaline. Euler ('12) states in a general way that peptases require a neutral or faintly alkaline substrate, and proteinases (tryptases) an acid one.

Of the proteins employed, solutions of casein and peptone prove the most favorable substrates. Albumin in solution is acted upon slowly, but when employed in the form of Mett's tubes, doubt exists regarding its digestion. Legumin appears to be slowly hydrolysed by *Enteromorpha* and *Chondrus*, but not by *Mesogloea*. Gelatin, either in the liquid state or in the form of Mett's tubes, is not attacked. As groups, the "reds" appear more active in proteolysis than do the "greens," while, as was true for carbohydrases, the "browns" show the least activity.

THE AMIDASES

The tissues from the several algae were tested for their ability to split NH_3 from such amino and amido compounds as urea, acetamid, asparagin, and methyl amine. These compounds were used in 1 per cent concentrations. Series were set up in which 50 cc. of the substrate to be tested were placed in flasks together with 2 grams of the powdered tissue and chloroform-thymol as an antiseptic. Checks were used with the nitrogen compounds alone and with the algal tissue in distilled water. The flasks of duplicate series were kept at room temperature and at 35°C. respectively, for 30 days, at the end of which time Folin's method was employed for the determination of any NH_3 that might have been split off. In the collection of the NH_3, Friedrich's improved gas washing bottles containing 250 cc. of N/50 HCl were used. Air was bubbled through by means of a suction pump for two hours, then 25 cc. portions of the collection acid were removed and titrated against N/50 NaOH, with alizarin red as an indicator. In no case was there any action over that evidenced by the checks.

These results are extremely interesting, in the case of *Ulva* especially. This form, as has been shown, thrives in waters where the organic nitrogen content is high. The question would at once arise whether this increased growth were due to the ability of the *Ulva* to break down the protein molecule and thus obtain an increased supply of nitrogen as NH_3, or whether it were due to the activities of the denitrifying bacteria rendering available a larger assimilable supply. That such bacteria are relatively abundant in sewage-contaminated water has been shown in the review of literature. We can conceive of another factor entering in—that of selective formation of enzymes. It might well be that with plenty of the amino-nitrogen available through the activity of bacteria, no amidases would be formed. The possibility of shedding some light on this point led to the experiments following.

Experiments on amidase formation by Chlamydomonas.—*Chlamydomonas* was grown in pure culture upon two different media; one (with one or two modifications, that used by

Schramm ('14)), containing $(NH_4)_2SO_4$ as a source of nitrogen, the other with nitrogen supplied as peptone and asparagin. These media complete were as follows:

A.		B.	
Agar	10.00 grams	Agar	10.00 grams
$(NH_4)_2SO_4$	.25 grams	Peptone	4.00 grams
$MgSO_4.7\ H_2O$	.10 grams	Asparagin	1.00 grams
K_2HPO_4	.10 grams	$MgSO_4.7\ H_2O$	.10 grams
$FeSO_4$	trace	K_2HPO_4	.10 grams
Glucose	10.00 grams	$FeSO_4$	trace
Distilled H_2O	500.00 cc.	Glucose	10.00 grams
		Distilled H_2O	500.00 cc.

These media, designated "A" and "B," were placed in 125 cc. Erlenmeyers, about 25 cc. to each flask, and the flasks placed horizontally until the agar hardened. A relatively large surface was obtained in this way and the harvesting of the alga later was facilitated. In inoculation, the alga was smeared over the surface of the agar to give an even growth. After a growth of thirty days, it was harvested by scraping from the agar surface with a stiff, platinum needle. The cells were then dehydrated with alcohol, acetone and ether, dried, and ground with an equal weight of fine quartz sand. Flasks were set up in duplicate in Wollf wash bottles, using 1 per cent asparagin as a substrate, with an amount representing .35 grams of sand-free algal powder. Checks were run on both the asparagin and the algal powder alone. One-half the series was taken down at the end of 7 days, the other half at the end of 15 days, and the NH_3 split off determined by the Folin method previously employed. The flasks were kept at a temperature of 35° C. The results are given in table XVI.

TABLE XVI
THE ACTION OF DEHYDRATED CHLAMYDOMONAS CELLS UPON ASPARAGIN

Substrate 50 cc. 1 per cent	Weight algal powder	Nitrogen as NH_3 in 50 cc. substrate mgms.			
		7 days	Net N	15 days	Net N
Asparagin	.35 grams "A"	.36	.16	.45	.21
	.35 grams "B"	1.17	.96	1.84	1.60
	.....	.18	.....	.22	.....
Water	.35 grams "A"	.02	.....	.02	.....
	.35 grams "B"	.03	.....	.04	.....

The amount of nitrogen in the checks is so small as to be well within experimental error. The NH_3 split off by powder

"B" would be almost negligible were the findings not so consistent. There is a definiteness about the increase over the checks that can hardly be ignored. In order to get further evidence on this point, however, another series (table XVII) was set up, using urea and asparagin in 1 per cent concentrations as substrates. The flasks were maintained at a temperature of 35°C. for 30 days.

TABLE XVII

THE ACTION OF DEHYDRATED CHLAMYDOMONAS CELLS UPON ASPARAGIN AND UREA

Substrate	Weight algal powder	Nitrogen as NH_3 split off in 30 days mgms.	Net nitrogen mgms.
Asparagin	.5 grams "A"	1.15	.20
	.5 grams "B"	3.10	2.20
	.....	.65	.....
Urea	.5 grams "A"	1.45	.17
	.5 grams "B"	3.70	2.47
	.....	.98	.....
Water	.5 grams "A"	.30	.....
	.5 grams "B"	.25	.....

In this, as in table XVI, the evidence goes to show that although the desamidization is practically negligible where the alga is grown with $(NH_3)_2SO_4$ as a source of nitrogen, it is definite where the nitrogen is supplied in the amino and amido form. The actual splitting is small in any case.

On the basis of the above, we can simply reason by analogy, and yet this analogy points to the fact that the probable reason for the failure to demonstrate amidase in *Ulva* lies in the failure to form that enzyme. This in turn would indicate that the great growth of *Ulva* in sewage-contaminated waters is probably due to the abundance of desamidizing bacteria which those waters maintain—bacteria which break down the protein molecule with the ultimate setting free of NH_3. Nitrogen, as such, becomes directly available to the plant.

NUCLEASES

The presence of nucleases in the algae has already been reported by Teodoresco ('12), but since he investigated only

one of the forms falling within the scope of this study, experiments were carried on to determine the presence or absence of nucleases in one representative of each group, *Ulva*, *Ceramium*, and *Ascophyllum*.

One-half per cent nuclein was dissolved in N 10 NaOH, the complete solution of the compound being shown by a drop of phenylphthalein. One hundred cc. of this neutral solution were added to each flask together with 3 grams of air-dried algal powder. Toluene was added as an antiseptic. Checks were set up by adding autoclaved algal powder to the nuclein and also by using nuclein solution alone. The flasks were placed at 35–36° C. for 38 days, at the end of which time the phosphoric acid split off was determined as P_2O_5 by the uranium-acetate method.[1] Five cc. of a sodium acetate solution[2] were added to 25 cc. of the nuclein substrate, this brought to a boil and titrated while hot. Potassium ferrocyanide was used as an indicator—a drop of the titration mixture being removed from time to time and brought into contact with a drop of the indicator on a porcelain plate. The results obtained are given in table XVIII.

TABLE XVIII

THE ACTION OF POWDERED TISSUE FROM CERTAIN ALGAE UPON NUCLEIN

Substrate 100 cc. 1 per cent	Weight algal powder	Free H_3PO_4 as P_2O_5 in 100 cc. in 38 days mgms.	Net amount P_2O_5 in 100 cc. mgms.
Nuclein	3 gms. *Ceramium*	70.00	56.25
	3 gms. *Ceramium* boiled	13.75	.....
	3 gms. *Ulva*	56.70	39.20
	3 gms. *Ulva* boiled	17.50	.....
	3 gms. *Ascophyllum*	17.25	.35
	3 gms. *Ascophyllum* bld.	16.90	.....

These findings substantiate those of Teodoresco ('12) regarding the general presence of nucleases in the algae. The values for *Ceramium* agree very well with those he obtained for the same form, i. e., 56.25 milligrams in 38 days at 35° C., as compared with 76.6 milligrams in 51 days at 22–26° C. *Ulva*

[1] The standard solution of the uranium acetate contained 8.8652 grams of the salt in 250 cc. of water, and each cc. by calculation was equivalent to 5 milligrams of P_2O_5.

[2] The sodium acetate solution contained 25 grams of sodium acetate and 25 cc. of 30 per cent acetic acid in 250 cc.

shows less nuclease activity than does *Ceramium,* while *Ascophyllum,* true to its reputation for inactivity, gives a value so small as to be negligible.

An interesting point is brought out by the use of nuclein—one that proves a check on some of the previous proteinase experiments. Nuclein is composed of nucleic acid bound up with some protein (according to Abderhalden, '11, this is albumin) which must be split off by a proteinase before the nuclein residue is exposed to the attack of the nuclease. That unmistakable nuclease activity was evident, only serves to show again the presence of proteolytic enzymes.

OXIDASES AND CATALASES

Oxidases.—Direct and indirect tests for oxidase action, that is, for the oxidases and the so-called peroxidases, were carried out in all cases with fresh tissue. The general method described by Clark ('10) was employed, using guaiacum, alpha naphthol, and phenylphthalin as reagents. Five grams of the fresh tissue, crushed with an equal weight of fine quartz sand, were extracted for half an hour with 25 cc. of distilled water. The extracting fluid was then filtered off, the tissue residue pressed out, and the filtrate made up to 50 cc. Five cc.-portions were placed in test-tubes, and for the direct test, ten drops of the reagent were added; for the indirect test, this amount plus 1 cc. of fresh 3 per cent hydrogen peroxide. In only two cases was direct oxidization observable—with *Agardhiella* and *Ulva.* With the former, direct action was strong with all three reagents, and when peroxide was added an immediate deepening of the color occurred, showing the presence of peroxidases as well. With *Ulva,* however, both direct and indirect tests were only weakly positive. Atkins ('14), it will be remembered, obtained direct tests with but one of twenty-nine diverse algae investigated and indirect tests with but seven. He thought that reducing substances prevent the demonstration of oxidases in other forms. As brought out in the review of literature, Reed ('15[a]) has since demonstrated indirect oxidation of the alpha naphthol-paraphenylenediamine group of compounds by many of these

forms. In the filamentous forms he showed the presence of oxidases by the formation of colored granules within the cells surrounded by these reagents. Reed concludes that oxidases of specific oxidative ability are very generally present in the algae, and where negative results are obtained, either the necessary specific compound is not present or other factors enter in, such as the destruction of the oxidase equilibrium of the cell upon crushing.

Catalases.—Both fresh and air-dried tissue were used for catalase demonstration. In a preliminary series, the addition of 5 cc. of 3 per cent hydrogen peroxide to about a gram of fresh crushed algal tissue showed evolution of oxygen in all cases except one, that of *Mesogloea*. Later, a series (table XIX)

TABLE XIX

CATALASE ACTIVITY OF CERTAIN ALGAE

Alga	Number cc. O_2 evolved at 21.5°C.		
	2 minutes	5 minutes	10 minutes
Ascophyllum	.3	.9	.9
Laminaria	1.4	2.3	2.3
Mesogloea	0.0	0.0	0.0
Ulva	.2	.4	.5
Agardhiella	3.3	4.6	5.6
Chondrus	.4	2.0	2.5
Rhodymenia	.9	1.4	2.0
Ceramium	3.7	5.9	8.2
Potato leaf tissue	22.6	....	....

was set up in which 1 gram of powder was placed in 125 cc. Erlenmeyer flasks, 10 cc. of 3 per cent hydrogen peroxide added, and the oxygen evolved collected in a gas burette over water. The flask in which the action was taking place was shaken every 15 seconds, and the volume of oxygen evolved read at the end of 2, 5, and 10 minutes. The temperature of the room was practically constant during the experiments and no especial precautions were taken to control the temperature of the flask other than keeping the hands away from it during the action. The results are not meant to be quantitatively exact, but they do give the relative catalase activity of the several forms. In addition, air-dried potato leaf tissue that had been in the laboratory about the same length of time as the algal tissue was tested for comparison.

Catalase, so wide-spread in all plant tissues, is found here in all the forms investigated except *Mesogloea.* The "reds" prove more active than the "browns," and these latter slightly more active than the "greens." No alga is strikingly active, however, when compared with potato leaf tissue. Strangely, *Ulva,* most active in regard to the other enzyme groups, is one of the least so here.

General Discussion and Conclusions

The data obtained in the foregoing investigation serve to show that the number of enzymes in the algae that can be isolated, by standard methods at least, is quite limited. This is especially true of the "browns," in two forms of which, *Ascophyllum,* and the *Fucus* of the earlier study, such action is limited to catalase alone. In this group the demonstrable carbohydrases are restricted to very slowly acting diastases in *Laminaria;* in neither *Ascophyllum* nor *Mesogloea* is there the slightest trace of what might be termed carbohydrate hydrolysis. Moreover, negative results are obtained in these forms for most of the other enzymes sought. *Laminaria* shows lipases and catalases (it was not tested for proteolytic or nuclease activity), and action in *Mesogloea* is restricted to lipases and proteinases, both tryptic and ereptic. On the other hand, very general enzymic activity is demonstrable in the "greens" and the "reds"—diastases, dextrinases, lipases, proteinases (tryptic and ereptic), nuclease, and catalase being isolated from the crushed tissue. Oxidase is shown present in one "red," *Agardhiella,* and in one "green," *Ulva.* Such action, as a whole, appears a little more rapid in the "reds" than in the "greens," but no enzyme stands out as being specific for either a group or an alga within a group.

The carbohydrases demonstrated are restricted in their action to those hydrolysing starch, dextrin, glycogen, and laminarin of the polysaccharides used as substrates, and in *Laminaria,* such action was further limited by a failure to act upon glycogen. In no case, in any member of the three groups was there evidence of disaccharides being attacked. While

this is not so surprising perhaps for sucrose and lactose, it is difficult to understand the failure of enzymic hydrolysis of maltose. The results obtained by Kylin ('13) indicate that both dextrose and fructose are found in algal tissues, and reasoning from results found for plant and animal tissues in general, it seems, as is true in those cases, that in the algae, maltose must be broken down to glucose before assimilation can take place. The failure to isolate this enzyme points to the possible presence of some inhibiting factor, rather than to the non-formation of the ferment.

Lipases, acting very slowly, appear wide-spread in algae, being demonstrable in all the forms used in this study excepting *Ascophyllum*. Along with the fact that fats are very generally found in the algae, these results are significant in that they indicate the importance of the rôle these compounds may play as assimilatory products. It is not thought, as was advanced by Reinke ('76), Hansen ('93), and others, that these fats function as the first products of assimilation, but rather, that they act as storage products of more or less importance.

The algae, in general, show the presence of enzymes capable of hydrolysing certain proteins. Casein and peptone in alkaline and neutral solution prove the most favorable substrates of those tested, although legumin and albumin are also slightly attacked. The "greens" and the "reds" are about equally active in this way, the "browns," as usual, acting more slowly. The fact that both native proteins and peptones were hydrolysed, points to the presence of both tryptic and ereptic enzymes. Still further evidence of the presence of the first of these was the splitting of the protein molecule from nuclein preceding the action of nuclease.

Amidases seem not to be formed by any of these algae. The results obtained with *Chlamydomonas*, from which the amidases were isolated when the alga was grown on a medium containing asparagin and peptone as a source of nitrogen but not when the nitrogen was in the form of ammonium sulphate, indicate that such amidase formation may depend upon the nature of the supply of assimilable organic nitrogen. This has a distinct bearing upon the reason for the increased growth of

Ulva in sewage-contaminated waters. In order to break down the proteins present in the surrounding waters and even those in close contact with the plant itself, it would be necessary for the *Ulva* to secrete an extracellular enzyme, since the large protein molecule is not diffusible into the cell. If so secreted, the enzyme would be quickly dissipated in the large volume of surrounding water. Desamidizing bacteria, on the other hand, have been demonstrated in harbor and shore waters where such algae abound. They can come into much more intimate contact with the protein than can the plant, and undoubtedly play an important rôle in rendering available at all times an abundant supply of organic nitrogen.

The demonstration of nucleases acting upon the previously split nuclein molecule, substantiates the findings of Teodoresco for this enzyme. Both *Ulva* and *Ceramium* showed the presence of the ferment, while *Ascophyllum,* the only representative of the "browns" investigated, gave negative results. Where such enzymes were formed, they compared more favorably with enzymes of fungi and higher plants than do any of the other algal ferments.

None of the "browns" studied showed the presence of oxidative enzymes, while in the "reds" and the "greens" but one form gave the characteristic reactions. It is interesting to note that these algae, *Agardhiella* and *Ulva,* were the most enzymatically active forms studied. The oxidase reactions with guaiacum, alpha naphthol, and phenylphthalin were very positive, both directly, and indirectly with hydrogen peroxide.

In all cases where enzymes were demonstrated, the action was very slow, being with the exception of nuclease, much less rapid than in the higher plants. The reason for this is not clear, but it cannot in all instances be due to inhibiting substances set free upon the death of the cell. Arber ('01), as has been mentioned before, found that *Ulva, Cladophora,* and *Enteromorpha,* placed in the dark but under otherwise presumably normal conditions, required from two weeks with *Ulva,* to two months and more in the case of *Enteromorpha* for destarching. This indicates the presence of a very slowly acting diastase in the cells of these algae. The metabolism

of the algae is also probably slower than that of the higher plants and one might expect, *a priori,* the enzymes also to be less rapid in their action. Although the algal enzymes may be inherently slow, it seems that there may also be substances set free on the death of the cell which either partially or entirely inhibit enzyme action. The writer has found evidence in some preliminary experiments, that the action of taka diastase upon starch is directly proportional to the amount of free tannin present. In connection with this, it was also found that *Ascophyllum* had a "tannoidal" content of 1.1 per cent of the dry weight. It is possible that such tannoids, if in an uncombined state, may after the death of the cell unite with an enzyme to throw it out of the sphere of action. That diastases are demonstrable in tissues having a high tannin content may perhaps be explained on the basis that they are bound up in such a way as to render them incapable of uniting with the ferments. Still other organic inhibiting compounds may be present, and the point opens up a very interesting problem concerning inhibition, not only in algal tissues, but in those of many higher plants as well.

Summary

1. Using standard methods of enzyme isolation and determination, the following enzymes have been found in fresh or dried algal tissue:

a. Carbohydrases hydrolysing the polysaccharides, starch, dextrin, glycogen, and laminarin, but not those hydrolysing the several disaccharides employed as substrates.

b. Lipases acting upon neutral fats but not upon the esters of the lower fatty acids.

c. Proteinases (tryptic and ereptic) acting best under neutral and alkaline conditions.

d. Nucleases.

e. Oxidases and peroxidases (in but two forms—*Agardhiella* and *Ulva*).

f. Catalases.

2. Negative results were obtained for cellulase, cytase, maltase, lactase, sucrase, amidase, and esterase.

3. The action of all the enzymes isolated was very slow.

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Xylophylla **Epiphyllanthus**, 42